Conclusions: Our data further demonstrate the complex genetics associated with leiomyosarcomas. As a class, tumor suppressor genes were the most commonly mutated loci, including *TP53,* mutated in 36% of cases (9/25). The loss of key tumor suppressor genes is consistent with our observations that CNV was present in 85% of leiomyosarcomas. While no common therapeutically targetable gene was identified across these cases, future large cohort sequencing studies of leiomyosarcomas may provide a means for the molecular classification of these tumors and identify new treatment paradigms.

107 Identification of a Novel FN1-FGFR1 Genetic Fusion as a Frequent Event in Phosphaturic Mesenchymal Tumor

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Background: Phosphaturic mesenchymal tumors (PMT) are unusual soft tissue and bone tumors that typically cause hypophosphatemia and tumor-induced osteomalacia through secretion of phosphatonins such as fibroblast growth factor 23 (FGF23). PMT has recently been accepted by the World Health Organization as a formal tumor entity. The genetic basis and oncogenic pathways underlying its tumorigenesis remain obscure.

Design: Four PMT samples were subjected to RNA sequencing in search of possible fusion transcripts, which would be confirmed by genomic DNA PCR, RT-PCR and western blotting. Fluorescence *in situ* hybridization (FISH) was performed on a total of 15 cases to check the presence of the fusion gene.

Results: Three of the four cases were found harboring a novel *FN1-FGFR1* fusion gene by RNA sequencing, which was confirmed by DNA PCR and RT-PCR. Western blotting corroborated the presence in two cases of the chimeric FGFR1 protein with increased sizes. FISH showed 6 cases with *FN1-FGFR1* fusion, out of an additional 11 PMTs. Overall, 60% (9/15) harbored this fusion.

Conclusions: We for the first time identified a highly recurrent genetic event in PMTs. The *FN1-FGFR1* fusion gene likely has an important role in tumorigenesis and may also have potential therapeutic implications. The *FN1* gene possibly provides its constitutively active promoter and the encoded protein's oligomerization domains to over-express and facilitate the activation of the FGFR1 kinase domain. Interestingly, the FN1-FGFR1 chimeric protein, with various fusion points, was predicted to preserve its ligand-binding domains, suggesting an advantage of the presence of its ligands (such as FGF23) in the activation of the chimeric receptor tyrosine kinase, thus effecting an autocrine or paracrine mechanism of tumorigenesis. Further study is required to confirm these hypotheses.

108 A Subset of Spindle Cell Lipomas Harbors Rearrangements of the HMGA1 Locus

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Background: Spindle cell lipomas are benign adipocytic neoplasms composed of varying amounts of mature adipose tissue, bland spindle cells, and collagen fibers. A single case of spindle cell lipoma with t(2;6)(p16~21;p21) expressing HMGA1 protein was reported by Dumollard JM et al in 2001. We have encountered a second case with classic histologic features and t(1;6)(p32;p21.3). This finding led us to hypothesize that, similar to ordinary lipomas, a subset of spindle cell/pleomorphic lipomas could also harbor rearrangements of the chromatin remodeling gene *HMGA1*.

Design: Twenty-four spindle cell lipomas and 8 pleomorphic lipomas with classic histologic features were retrieved from our institutional archives and screened for rearrangements of the *HMGA1* and *HMGA2* loci using custom-designed break apart FISH probes. The spindle cell lipomas were seen in 20 males and 4 females with a mean age of 59 years (range 40-86 years) and involved most frequently the neck (7), back (7), face/ear (3), arm/shoulder (2), thigh (2), buttock (2), and labia (1). The pleomorphic lipomas were seen in 7 males and 1 female with a mean age of 56 years (range 37-72 years) and involved the shoulder/neck (4), ear/scalp (3) and back (1).

Results: Balanced rearrangements of the *HMGA1* locus were found in 3 (of 24; 13%) spindle cell lipomas but in no pleomorphic lipoma. All tumors were negative for *HMGA2* rearrangements.

Conclusions: Similar to ordinary lipomas, a small subset of spindle cell lipomas harbors rearrangements of the *HMGA1* locus. This finding suggests that these two subtypes of lipoma may share common oncogenic pathways, at least in a subset of cases. Further studies are needed to better understand the biologic implication of this novel finding in spindle lipoma pathogenesis.

109 Morphologic Diversity in Desmoid-Type Fibromatosis: Clinico-Pathologic Correlation and Potential Diagnostic Pitfalls on Core Biopsy Specimens

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Background: Desmoid-type fibromatosis is a locally aggressive neoplasm characteristically composed of long sweeping fascicles of bland fibroblasts and myofibroblasts that may occur at a variety of abdominal, intra-abdominal and extra-abdominal sites. Occasionally alternative morphologic patterns are identified, and this may lead to diagnostic difficulty on small biopsy specimens, especially for pathologists who do not routinely examine soft tissue specimens. Additionally, little data is available as to the distribution and frequency of these patterns.

Design: 165 resection specimens of desmoid-type fibromatosis were retrieved from our institutional archives. All available H&E slides from each case (ranging from 1-14 slides) were reviewed, and the diagnosis was confirmed. The morphologic patterns were catalogued and compared by site, age and sex.

Results: Patterns identified included: conventional (165 cases, range 10-100%), hypocellular/hyalinized (46 cases, range 5-80%), staghorn vessels (35 cases, range 5-30%), myxoid (27 cases, range 5-40%), keloid (24 cases, range 5-50%), nodular fasciitis-like (15 cases, range 5-40%), and hypercellular (6 cases, range 5-20%). Tumors with keloid areas, as well as those with prominent staghorn blood vessels, mimicked entities such as solitary fibrous tumor. Those cases with nodular fasciitis-like areas raised the possibility of nodular fasciitis and reactive processes. Hypercellular foci led to the consideration of spindle cell sarcoma, while the differential diagnosis of hypocellular/hyalinized areas was broad. By site, the greatest variation of patterns was observed in intra-abdominal lesions, and men showed more morphologic variability than females. Adults (>18 years) exhibited more histologic diversity than adolescent and pediatric patients (≤ 18 years).

Conclusions: The morphologic spectrum of desmoid-type fibromatosis is diverse and often underappreciated. Intra-abdominal lesions tend to show the greatest morphologic diversity, and awareness of the histologic patterns that may occur is necessary to prevent misdiagnosis, especially on small biopsy specimens.

110 Myxoinflammatory Fibroblastic Sarcoma (MIFS) and Hybrid Hemosiderotic Fibrolipomatous Tumor (HFLT)/ MIFS: Related or Not? A Clinicopathological and Molecular Cytogenetic Study of 34 Cases

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Background: MIFS, a locally aggressive, rarely metastasizing fibroblastic tumor that typically involves the distal extremities, has been reported to show t(1;10)(*TGFBR3-MGEA5*) in some instances. This genetic event has also been reported in HFLT, and in rare tumors showing hybrid features of HFLT and MIFS. These findings have led to speculation that HFLT and MIFS are closely related. However, areas resembling HFLT have been present in only a very small minority (~1%) of previously reported MIFS, and we recently found *TGFBR3* or *MGEA5* rearrangements in only 1 of 6 studied cases. Thus the relationship between MIFS and HFLT is still unclear. We studied the clinicopathological and molecular cytogenetic features of 34 cases of MIFS and hybrid HFLT/MIFS with the goal of clarifying these issues.

Design: Available slides from 38 cases diagnosed as "MIFS" or "hybrid HFLT/MIFS" were retrieved from our archives. Following re-review by 2 experienced soft tissue pathologists, 4 cases were excluded, leaving a final group of 27 MIFS and 7 hybrid HFLT/MIFS. Cases were tested for *TGFBR3* and *MGEA5* gene rearrangements using previously published methods.

Results: MIFS occurred in 10F and 17M, with a mean age of 45 years (range 15-82 years) and involved the hand/arm (N=12), foot/leg (N=14), and cheek (N=1). Hybrid HFLT/MIFS occurred in 6F and 1M, with a mean age of 60 years (range 49-78 years) and involved the foot (N=4) and leg (N=3). All MIFS conformed strictly to the current WHO definition; no case showed areas resembling HFLT. Hybrid HFLT/MIFS showed zones of classical HFLT juxtaposed to areas of myxoid sarcoma, showing some but not all features of classical MIFS. By FISH, 11 informative MIFS were negative for *TGFBR3* or *MGEA5* rearrangements. In contrast, 2 of 4 tested hybrid HFLT/MIFS showed *TGFBR3* or *MGEA5* rearrangement.

Conclusions: Our morphological and FISH findings suggest that MIFS and hybrid HFLT/MIFS may be unrelated, with the latter entity representing a form of morphological progression within HFLT. We speculate that not all studies may have utilized the same diagnostic criteria for MIFS. On-going FISH study of the remaining cases should help to clarify these issues.

Breast Pathology

111 Multicenre Genomic and Protein Expression Analysis Reveals SPAG5 as a Key Oncogene and Biomarker and a Target for Personalized Therapy in Breast Cancer (BC)

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Background: Recently our neural network analysis of Breast Cancer gene expression array (GEA) data has revealed SPAG5 as a major hub in the proliferation pathway. In this study pre-clinical, molecular and clinicopathological functions of SPAG5 was investigated in patient cohorts from multi-centres.

Design: 1. The expression and functionality of *SPAG5*, its relationships to the p53 network and the response to different therapies, have been evaluated pre-clinically in a panel of BC cells.

2. Gene expression analysis of mRNA SPAG5 and its clinicopathological significance have been analysed in two large cohorts (METABIRIC cohort; n=1950 and Uppsala cohort; n=249), and then validated in a 3400 case dataset derived from global multi-centre resource. Neural network and functional pathway analysis have been carried out in 2000 BC cohort.

3. The relationships between SPAG5 protein expression and clinicopathological outcomes and response to systemic therapies have been analysed in 2500 BC patients derived from four cohorts including: 1) a series of 1650 primary early BC cohort, 2) 350 ER negative BC cohort treated with adjuvant anthracycline combination therapy, 3) 250 locally advanced BC treated with neoadjuvant anthracycline with/without taxane, and 4) 250 of HER2 positive BC treated with trastuzumab based adjuvant CT.

4. A series of 171 BC was used for integrative analysis of SPAG5 gene copy number (using aCGH), mRNA expression (using GEA) and protein expression.

Results: 1. 5% and 15% of BC showed amplification and gain of SPAG5 locus, respectively, at 17q11.2. SPAG5 mRNA expression displayed a significant correlation with its copy number (p< 0.0001).
2. SPAG5 mRNA overexpression was significantly associated with high histological and molecular grade, high mitotic index, ER negative PR negative, TP53 mutation, lymphocytic infiltration, triple negative phenotype, PAM50.Her2, PAM50.Basal, PAM50.LumB, integrative molecular cluster 1 (intClust.1)), intClust.5, intClust.9 and intClust. 10 breast cancers (ps <0.0001). SPAG5 mRNA overexpression is associated with poor breast cancer specific survival in univariate and multivariate analysis (p<0.0001).
3. 30% of BC showed overexpression of *SPAG5* protein, and a significant association with aggressive phenotypes, high mitosis, ER-, high grade, p53 mutation and epithelial mesenchymal transition phenotypes (ps <0.0001). SPAG5 protein overexpression is associated with poor survival in univariate and multivariate analysis (ps<0.001). However, in ER- BC treated with adjuvant anthracycline, *SPAG5* positive (+) had lower risk of progression compared with SPAG negative (-) BC (p<0.0001). Similarly, *SPAG5*+ BC cases which received neoadjuvant chemotherapy achieved 38% pathological complete response (pCR) vs. 6% of *SPAG5*- cases (p<0.0001). After controlling for other predictors for pCR, *SPAG5* was an independent predictor (HR; 2.4; p=0.001). Preclinical, SPAG5 predicts response to therapy depending on TP53 mutational status.
Conclusions: SPAG5 is an important novel gene implicated in the survival of BC cells and its protein expression is an independent prognostic biomarker. The relationship between TP53 and SPAG5 can be utilized for developing a novel target therapy for BC.

112 Lysophosphatidylcholine Acyltransferase 1 (LPCAT1): A Novel Biomarker in Breast Cancer

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Background: In Egypt, breast cancer is the most common cancer in women. Alteration in lipid metabolism is an established hallmark of cancer cells. Lysophosphatidylcholine acyltransferase 1 (LPCAT1), a key enzyme in Lands' cycle, is recently implicated in carcinogenesis. Since published literature lacks comprehensive reports on LPCAT1 expression in breast cancer, the present study was carried out.
Design: LPCAT1 protein immunohistochemical expression was assessed in 80 primary invasive breast carcinomas, 24 metastatic lymph nodes, and 30 non-neoplastic breast tissue specimens. The relation between LPCAT1 expression and clinicopathological variables, hormonal receptor and HER2 status, molecular subtypes, and proliferative activity (Ki67) was statistically analyzed.
Results: LPCAT1 was significantly overexpressed in invasive breast carcinoma compared to non-neoplastic breast tissue (p= .000) with a statistically significant ascending pattern (p=.000) being the lowest in normal breast tissues, relatively increased in non-proliferative and proliferative fibrocystic disease, and the highest in primary invasive carcinoma. LPCAT1 expression was significantly higher at tumor's advancing edge compared to tumor center (p<.001) and correlated positively with tumor's grade (p<.001). Compared to primary tumor, LPCAT1 expression was significantly lower in adjacent ductal carcinoma in situ (p=.000) and significantly higher in matched metastatic lymph nodes (p=.000).
LPCAT1 overexpression was significantly associated with advanced TNM stage (p<.001), larger tumor size (p=.002), higher lymph node stage (p<.001), higher number of positive lymph nodes (p=.000), presence of distant metastasis at diagnosis (p=.004), higher proliferative activity (p=.000), negative ER, PR status(p<.001 and p<.001 respectively), positive HER2 status (p=0.002) as well as triple negative and HER2 disease molecular subtypes compared to luminal A subtype (p=.001 and p=.013 respectively).
Conclusions: LPCAT1 exerts an important, albeit not fully understood, role in breast cancer oncogenicity, possibly via alterations in lipid profile.
LPCAT1 is implicated in the evolution and progression of breast cancer and appears to play a crucial role as a determinant of local invasiveness and metastasis.
LPCAT1 represents a novel biomarker that reflects underlying biological alterations and thus constitutes a potentially promising target for new therapeutic strategies.

113 Can We Predict the Oncotype DX Recurrence Score? A Clinicopathologic Analysis of Discrepant Cases as Determined By Two Equations With Clinical Outcome

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Background: Oncotype DX (ODX) is an RT-PCR based 21-gene molecular assay validated to provide prognostic and predictive information in patients with estrogen receptor (ER) positive invasive breast cancers (BC). The ODX Recurrence Score (RS) is divided into three risk categories as low (<18), intermediate (18-30) and high (>30) risk of distant recurrence at 10 years. While recent studies have shown that RS can be predicted by equations incorporating standard histologic and immunophenotypic tumor characteristics, such as the "original Magee equation" (M0) and "Magee equation 2" (M2), there still remain a significant number of cases classified differently by these tests. We analyzed the discrepant cases with regards to clinicopathologic features and outcome.
Design: The study included 832 patients with ER positive BC who underwent ODX testing between 2003-2014. The histopathologic features of tumors were prospectively determined without knowledge of the RS results. The clinicopathologic features and outcome of cases classified concordantly versus discordantly by RS and M0 and M2 were compared.
Results: 461 (55.4%), 565 (67.9%) and 444 (53.4%) cases were classified as low risk by RS, M0 and M2, respectively. 292 (35.1%), 205 (24.6%) and 344 (41.3%) were intermediate, while 79 (9.5%), 62 (7.5%) and 44 (5.3%) were classified as high risk, respectively. M0 and M2 agreed with risk estimation by RS in 594 (71.4%) and 563 (67.7%) cases overall, while the risk was over- and under-estimated in 7.2%, 14.9%, and 21.4%, 17.4% of cases, respectively. Cases classified discordantly by RS vs M0 and M2 showed correlations with tumor size, histologic type and grade, mitotic activity, lymphatic invasion (M0 only) and ER and PR histologic scores, but not with ER and PR ODX scores. Cases classified as intermediate risk by RS but low risk by M0, showed significantly worse recurrence-free and distant recurrence-free survival compared to cases classified concordantly as low risk. No such difference was found in the case of M2.
Conclusions: Although the M0 and M2 equations based on routine histopathologic parameters may be used to predict RS, compared to M0, RS appears to identify some cases with increased risk of recurrence compared to cases classified by both tests as low risk. No such difference in outcome was seen in the case of M2, which appears to be a more reliable test to predict RS with regards to clinical outcome.

114 Prediction of the Oncotype DX Recurrence Score: Prospective Validation of Two Equations With Clinicopathologic Analysis and Outcome

Geza Acs, Nazanin Khkapour, John Kiluk, M Lee, Christine Laronga. Ruffolo Hooper & Associates, Tampa, FL; Moffitt Cancer Center, Tampa, FL.
Background: Oncotype DX (ODX) is an RT-PCR based 21-gene molecular assay validated to provide prognostic and predictive information in patients with estrogen receptor (ER) positive invasive breast cancers (BC). The ODX Recurrence Score (RS) is divided into three risk categories as low (<18), intermediate (18-30) and high (>30) risk of distant tumor recurrence at 10 years. Two recent studies have shown that RS can be predicted by equations incorporating standard histologic and immunophenotypic tumor characteristics. Our aim was to validate two such equations [referred to as the "original Magee equation" (M0) and the "Magee equation 2" (M2)] in a prospective dataset.
Design: The study included 832 patients with ER positive BC who underwent ODX testing between 2003-2014. The histopathologic features of tumors were prospectively determined without knowledge of RS results. Risk estimations by the three methods, as well as clinicopathologic features and outcome of cases classified as low, intermediate and high risk by RS, M0 and M2 were compared.
Results: 461 (55.4%), 565 (67.9%) and 444 (53.4%) cases were classified as low risk by RS, M0 and M2, respectively. 292 (35.1%), 205 (24.6%) and 344(41.3%) were intermediate, while 79 (9.5%), 62 (7.5%) and 44 (5.3%) were classified as high risk, respectively. A highly significant correlation was found between RS and M0 and M2 scores ($r = 0.5915$ and $r = 0.6525$, respectively, $p < 0.0001$ for both). M0 and M2 agreed with risk estimation by RS in 594 (71.4%) and 563 (67.7%) cases, with kappa values of 0.5370 and 0.4807, respectively. RS, M0 and M2 all showed significant correlations with histologic type and grade, mitotic activity, lymphatic invasion, Nottingham Prognostic Index, ER, PR and HER2 expression. Cases classified as low, intermediate and high risk by RS, M0 and M2 showed significantly different recurrence-free and distant recurrence-free outcome for all three tests. There was no significant difference in outcome between cases classified into similar risk categories by the three tests.
Conclusions: The M0 and M2 equations based on routine histopathological parameters can be used to predict RS, and all three tests can classify BC into groups with different clinical outcomes. Although the equations may be further refined and further validated, they may serve as surrogate markers for RS and may be judiciously utilized in making treatment decisions in select patients.

115 Retraction Clefts and Reversal of Cell Polarity in Metastatic Carcinoma in Sentinel Lymph Nodes Predict Additional Nodal Disease in Breast Cancer

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Background: The accuracy of sentinel lymph node (SLN) biopsy for predicting the status of axillary lymph nodes in clinically node negative invasive breast cancer (BC) patients has been confirmed. While patients with negative SLN need no further axillary surgery, for patients who have positive SLNs, completion axillary dissection (CALND) still remains the standard practice in most cases. Although the SLNs are the only positive nodes in 50-65% of these patients, currently no reliable means exist to identify patients who can be spared the potential morbidity of CALND. We have previously described that the extensive presence of retraction clefts (RC) and even focal reversal of cell polarity (RCP) in BC highly significantly correlate with lymphatic tumor spread. The aim of this study was to examine whether the presence and extent of RC and RCP in metastatic carcinoma in SLN can predict additional nodal disease.
Design: One hundred twenty-three consecutive cases of BC with 1-3 positive SLNs were selected for the study. The presence and extent of RC were determined in metastatic tumor deposits in SLNs. RCP was defined as prominent linear EMA immunoreactivity on at least part of the periphery of metastatic tumor cell clusters. Any presence of RCP was considered positive. The presence and extent of RC and RCP were correlated with clinicopathologic tumor features and the presence of additional nodal metastasis in CALND.
Results: SLN metastasis was confined to 1 SLN in 99 (-80%) cases and involved 2-3 SLNs in in 24 (20%) cases. 117 (95%) cases showed macrometastases, while micrometastases were seen in 6 (5%) cases. The extent of RC and the presence of RCP in positive SLNs showed significant correlations with the size of metastatic deposits in SLN, histologic tumor grade and the presence of lymphatic invasion, but not with patient age, tumor size, ER, PR and HER2 status. Additional nodal disease in CALND was seen in 60 (49%) cases. Both high level of RC and presence of RCP in positive SLNs significantly correlated with additional nodal disease ($p < 0.0001$ for both). Using a cutoff of 20% for the extent of RC, the sensitivity and specificity of RC and RCP to predict additional nodal disease were 0.5500, 0.8571 and 0.8000, 0.8193, respectively.
Conclusions: These results support the hypothesis that the presence of extensive RC and RCP play a significant role in the lymphatic spread of breast cancer. Assessment of RC and RCP in positive SLNs may help in selecting patients who can be spared CALND and its associated potential morbidity.

116 BRCA1/2 Status and Low Molecular Weight Cyclin E (LMWE) Expression in Triple Negative Breast Cancer (TNBC) as a Function of Race and Survival

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Background: Studies showed that women of African descent tend to have more triple negative type of breast cancer than other population of women. Also, studies showed higher proliferative activity in breast cancer of African American women as compare to Caucasian women which might have contributed to poor prognosis of breast cancer in African America women. High expressions of cyclin E mRNA have been implicated in BRCA1/2 associated breast cancers and it appears as low-molecular weight cyclin-E isoform. Also, studies of incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer indicated correlation between these two factors. Therefore, we propose that combined effect of BRCA status and low molecular weight cyclin-E (LMWE) expression play a synergistic role in the treatment response and survival of TNBC in different racial groups.

Design: 1236 breast cancer cases from M.D Anderson Cancer Center were reviewed for immuno-stained for low molecular weight cyclin-E(LMWE), ER, PR and HER2 expressions. All clinical information, pathology and demographic retrieved from computerized database. Tissue samples categorized based on patients' self-reported ethnicity. Cyclin E protein levels determined using affinity-purified polyclonal cyclin E antibody. Scoring of cyclin E IHC was subjective interpretation of staining in cytoplasm and nucleus of tumor cell that are positive. Survival rates calculated from the date of primary surgery until death from breast cancer or recurrence. Survival curve determined by Kaplan-Meier method and the significance will be assessed using log-rank test. The significance was assessed at the 5% level. The 95% Cl constructed and all statistical tests were two-sided.

Results: There is a difference in the disease recurrence rate within racial groups based on the whether cyclin E expression in cytoplasm or not was observed. African American patients have significantly higher TNBC and higher cyclin E expression than white with higher proportion of the expression occurred in the cytoplasm.

Conclusions: There is statistically significant difference in the disease recurrence rate within racial groups based on the cyclin E phenotype .Those with with cytoplasmic staining of cyclin E have higher disease recurrence rate. Thus,LMWE is associated with poor survival. African American have poor survival than caucasians.

117 Multifocal Breast Cancer: Distinguishing Independent Tumor Foci From In-Transit Metastases

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Background: Multifocal breast cancers (MBC) have a poorer prognosis compared to matched unifocal breast cancers. Studies have suggested that this is due to tumor size underestimation in MBC. Our hypothesis is that a subset of MBCs behaves in a fashion analogous to 'satellite' and 'in-transit metastasis' observed in melanoma, and thereby, are more significantly correlated with lymphovascular invasion (LVI) and nodal metastasis.

Design: Multifocal breast cancer patients were identified within the past 3 years through a retrospective search of our pathology database for "breast", "multifocal", "multicentric", "foci" and "satellite." Routinely-stained glass slides from each case were examined. Cases were classified into two groups: study cases defined as ³2 morphologically similar tumor foci with at least one focus without in situ carcinoma; and control group defined as ³2 morphologically similar or dissimilar foci with associated in situ carcinoma in all foci. Cases of purely lobular carcinoma, recurrent carcinoma, treated carcinoma or microinvasive carcinoma were excluded.

Results: 69 cases of MBC were identified, of which 19 were classified as study cases and 50 were classified as control cases. Study cases had significantly greater tumor size (largest tumor and total cumulative size) and a significantly higher percentage of patients with LVI and nodal metastasis. See table 1.

	Study cases (n=19)	Control cases (n=50)	p
Largest Tumor size (median) cm	1.8	1.1	0.048
Cumulative size (median) cm	3.5	1.8	0.003
LVI %	95	18	
Nodal metastasis %	74	18	

In addition, a higher percentage of study cases presented with ≥5 tumor foci compared to controls (42% and 2%, respectively; p=0.0002). Among controls, the majority of patients had only 2 tumor foci (58%). There was a greater percentage of patients with grade III tumors among study cases compared to control cases (63% and 26%, respectively; p=0.0088). There was no statistical difference between the groups with respect to age and biomarkers.

Conclusions: There is an aggressive subset of MBC that often presents with 5 or more tumor foci/satellite nodules and a higher rate of nodal metastasis. The aggressive behavior of these cases may be cautiously attributed to their proclivity for LVI in a phenomenon akin to 'satellite' and 'in-transit metastasis' in melanoma.

118 Histogram of Hosoya Indices for Assessing Similarity Across Subgraph Populations: Breast Cancer Prognosis Prediction From Digital Pathology

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Background: Spatial arrangement of nuclei in digitized pathology images has been shown to be strongly associated with disease aggressiveness and patient outcome for a number of different cancers. There might be recurring structural patterns that are linked to disease outcome. Several researchers have shown that by constructing a graph network, built using nuclei as vertices may actually be a reflection of the underlying molecular biology of the tumor. What remains unexplored however, is identifying similar subgraph structures that are recurring across the population and their effect on overall tumor morphology. Hosoya index (HI) was originally introduced as a measure of chemical bonds. We sought to leverage HI to measure structural similarities of graphs (bonds) across the populations that are indicative of recurrence in breast cancer tissue images.

Design: Using a tissue microarray cohort of 74 early-stage estrogen receptor positive (ER+) breast cancer (BCa) patients (diagnosed with invasive ductal carcinoma) were divided into two groups corresponding to patients who experience lifetime distant recurrence (N=23) and those who did not (N=51). All TMA cores were digitized at 20x magnification (0.33 um/pixel spatial resolution). Each nucleus was identified via a computerized image analysis algorithm. Then, using a novel cell graph that encodes a link between a pair of nodes based on proximity, a series of graphs are constructed on a TMA. From each of the resulting graphs, a signature is created based on the distribution of HI value measured on each of the graph in the TMA core. A random forest classifier is developed, trained and validated on these signatures over 10 runs of 3-fold cross validation using subsets of the cases for independent training and testing. Our hypothesis was that there are recurring bonds (as measured by HI signature) across the patient population that is predictive of life-time recurrence in BCa histopathology.

Results: For the ER+ BCa dataset, our method was able to predict recurrence with an accuracy of 79.7%.

	ER+BCa (n=74)
Accuracy	79.7± 0.6
PPV	82.6± 0.2
NPV	78.4± 1.2

Conclusions: Based only on tiny H&E punches, a computer-aided classifier can strongly predict tumors at low likelihood of recurrence. With further validation, this may be a very useful in practice to select patients for de-escalated therapies versus those who should receive more aggressive treatment.

119 Histologic Features Associated With Diagnostic Disagreement in Atypical Ductal Hyperplasia of the Breast: Results From the B-Path Study

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Background: Overall diagnostic agreement is low for atypical ductal hyperplasia (ADH), but histologic characteristics associated with diagnostic agreement for ADH are poorly understood. The B-Path study, which is looking at issues in diagnostic reproducibility in breast pathology using 240 cases with expert consensus diagnoses and interpretations from 115 participating pathologists, presents a uniquely well-characterized set of cases to examine features associated with diagnostic agreement.

Design: The 72 B-Path test cases with a consensus diagnosis of atypia were reviewed digitally for categorization of the following features: specimen type, low power screening features, presence of additional potentially distracting diagnoses, differential diagnosis within the lesion of interest, extent of atypia present (number of separate areas, number of foci within an area, size of largest area and focus), architectural patterns present within the atypia, cytology (very vs. borderline monotonous), and uniformity of architectural changes throughout the lesion. These features were then correlated with the mean percent participant agreement on the consensus diagnosis of atypia.

Results: Overall diagnostic agreement of participants with the consensus diagnosis of ADH was 48%. Factors associated with differences in agreement included non-cribriform architectural growth pattern (lowest agreement, 39%, when non-cribriform), solid/subtle architectural development (lower agreement, 21%), atypia within a papillary lesion (higher agreement when papillary, 56%), > 5 regions of interest to examine (lower agreement, 44%), cytologic monotony obvious vs borderline (higher agreement when obvious, 50%), and only 1-2 foci of atypia present (lower agreement, 44%). Lower agreement cases were notable for having focal, subtle ADH that was borderline with usual ductal hyperplasia or had a solid growth pattern that raised the differential of a lobular lesion. Higher agreement cases were notable for larger areas with ADH, frequently involving a papillary lesion.

Conclusions: Not all atypia diagnoses have the same diagnostic reproducibility. Given the poor agreement on cases of ADH that are focal and subtle, these cases should not be over-treated. Atypia is a more reproducible diagnosis when it is larger, non-focal or papillary.

120 Hematologic Malignancies of the Breast

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Background: Hematologic malignancies of the breast are extremely rare. Breast lymphoma represents only 0.04-0.5% of malignant breast tumors, and breast myeloid sarcoma and plasmacytoma are so rare that data exist mainly as case reports. Diagnosis is challenging in patients without a history of hematologic neoplasms, especially since primary breast carcinoma is usually the first clinically suspected entity.

Design: We conducted a retrospective review at our institution's pathology database for the past 22 years (1992-2014) for hematologic lesions diagnosed in breast tissue. Clinical characteristics, prior history, histologic subtype, and patient outcomes were analyzed.

Results: We identified 58 patients; 57 females and 1 male, with mean age of 58 years (range 29-93 years). The cases included 52 lymphomas (20 diffuse large B-cell lymphoma, 13 follicular lymphoma, 7 extranodal marginal zone lymphoma, 3 anaplastic large T-cell lymphoma, 2 peripheral T-cell lymphoma, 2 small lymphocytic lymphoma, and one case each of Burkitt lymphoma, mantle cell lymphoma, precursor T-cell lymphoblastic lymphoma, B-cell lymphoblastic lymphoma and cutaneous T-cell lymphoma), 4 plasmacytomas secondary to multiple myeloma and 2 myeloid sarcomas. Seven patients (12%) presented with multiple breast tumors. Thirty-three patients (57%) had primary breast disease. Twenty-three (40%) had breast lesions secondary to a previously diagnosed hematologic malignancy, and average time for breast relapse was 5.5 years (range 1-23 years). Status was unknown in 2 patients (3%). Six of 58 patients (10%) had prior breast carcinoma. Four of the 33 patients with primary disease had prior chemotherapy, and 5 had a history of autoimmune disease. Follow up was available in 44 patients, including 38 patients with lymphoma: 35 were alive (follow-up time 1-13 years) and 9 died (1 day-4 years after diagnosis). Additionally, 5 of 38 (13%) had transformation to a higher grade lymphoma.

Conclusions: The most common hematological malignancy is DLBCL. A high percentage of patients (33/52, 63%) with breast lymphoma have primary disease (ratio of 2:1 of primary vs secondary). Five of these patients had a history of autoimmune disease, and 4 others had a history of prior chemotherapy. However, 21 patients with primary breast lymphoma (64%) had no known risk factors for hematologic malignancy. Therefore, a higher level of clinical suspicion for lymphoma is warranted in patients with primary breast tumors.

121 Impact of the 2013 HER2 Guidelines Recommendations By FISH, Retrospective Analysis of 1893 Cases at Fundación Santa Fe de Bogotá

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Background: Human epidermal growth factor receptor 2 (HER2) is a member of the erbB class tyrosine kinase receptor and is amplified or overexpressed in 15% to 20% of primary Breast cancer (BC). HER2 oncogene overexpression is intimately related with unregulated cell proliferation and results in an aggressive BC. The need for and updated ASCO/CAP guideline on accurate HER2 testing was directed to ensure a correct treatment, and 2013 guideline emphasized in changes that would mitigate false positives, particularly relating to issues of specimen fixation and pathologist interpretation. The aim of this study was to present our experience in HER2 testing by FISH according to 2007 and 2013 ASCO-CAP recommendations.

Design: We reviewed all HER2 test studies by FISH in BC between the years 2010 and 2013 according to 2007 and 2013 ASCO-CAP *HER2 guidelines recommendations by FISH*. Cases were grouped according to; indeterminate, positive and negative categories.

Results: From the total 1893 HER2 cases studied by FISH, the initial 399 reported as positive remain positive, however total positives increased with the new guidelines in 416 cases due to new recommendation of ¨dual-probe *HER2*/CEP17 ratio ³2.0; with an average HER2 copy number <4.0 signals/cell and/or Single-probe average HER2 copy number ≥6.0 signals/cell¨. In relation with the 1458 samples classified as negative, diagnosis changed in 53 cases (3.6%): 50(3.4%) diagnosed as negative became undetermined and 3(0.2%) initially classified as negative turn out to be positive. Finally among the initial 36 cases classified as undetermined, 21 cases (58.3%) changed: 7(19.4%) became negative and 14(38.8%) positive.

Conclusions: With the implementation of the 2013 test guideline recommendations in BC, diagnostic category changed only in 74 patients (3.9%). No false positive cases were identified, which is consistent to international prospective studies where false positive test results have diminished. Unfortunately, we identified 3 false negative case that corresponded to 0.2% of the total cases classified as negative that could lead to denial of Trastuzumab treatment in a patient who could probably benefit from it, this scenario justifies one of the main reasons of accurate HER2 testing: ensure that the right patient receives the right treatment.

122 Imaging Characteristics of Invasive Ductal Carcinoma With Lobular Features

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Background: Invasive ductal carcinoma with lobular features (IDC-L) is not a recognized subtype of breast cancer, but a category used when there is a mixture of ductal and lobular morphologies. We have shown in prior studies that IDC-L may be a distinct variant of invasive ductal carcinoma (IDC), but have clinicopathologic features more similar to that of invasive lobular carcinoma (ILC), including higher frequency of multiple tumor foci and re-excision rates when compared to IDC. In this study, we sought to correlate the radiographic features of IDC-L with clinicopathologic features.

Design: 176 of 183 patients with IDC-L had mammograms, of which, 136 also had ultrasounds. Imaging features were recorded from the radiology reports and categorized as mass and non-mass findings which were subsequently correlated with the clinicopathologic features.

Results: Mass was the most common finding on mammogram (113/176, 64%) and ultrasound (121/136, 89%). 6 patients (3%) had a benign mammogram and 10 patients (7%) had a benign ultrasound. No association was seen between the percentage of lobular morphology and radiographic features or re-excision rates.

Multiple tumor foci were identified in 48% (27/56) of patients with multiple foci reported on pathology (6 on mammogram, 11 on ultrasound, and 10 on both). Of these 27 patients, almost half had a mastectomy (48%, 13/27). For the remaining 14 patients who underwent breast conserving surgery, 50% (7/14) required re-excision. Of the 29 patients who had undetected multiple tumor foci on imaging, 34% (10/29) had a mastectomy. All but 2 patients who had breast conserving surgery required re-excision (89%, 17/19).

Excluding patients who had mastectomies as their first procedure, re-excisions were more common in patients with non-mass findings on both mammogram (25/36, 69% for non-mass vs 40% for mass) and ultrasound (7/9, 78% for non-mass vs 37% for mass). For those with a mass on imaging, the size of the largest mass reported on mammogram and ultrasound was underestimated when compared to the reported size on pathology in 47/104 patients (45%) and 66/113 patients (58%), respectively. Re-excision was more common in patients with underestimated tumor size on mammogram (14/28, 50%) when compared to those who did not (16/44, 36%), but not on ultrasound, where re-excision was comparable (30% vs 37%).

Conclusions: Pre-operative imaging in patients with IDC-L often underestimates the size and presence of multiple tumor foci. This study suggests that both of these factors may contribute to the higher re-excision rates previously reported in this cohort of IDC-L patients.

123 Androgen-Related Tumor Suppressor NKX3.1 Is Expressed More Often in Invasive Ductal Carcinomas in Male Patients than in Female Patients

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Background: The androgen-related tumor suppressor NKX3.1 colocalizes with androgen receptor (AR) in prostate cancer and inhibits estrogen receptor (ER) signaling in breast cancer models. Immunohistochemistry (IHC) for NKX3.1 is largely specific for prostatic carcinoma and tissues but is also seen in breast carcinoma. We previously showed that NKX3.1 labeling in female breast carcinomas is correlated with lobular morphology, ER positivity and AR positivity. Specifically, NKX3.1 labeling is seen in 27% of female invasive lobular carcinomas (ILC), 2% of female invasive ductal carcinomas (IDC), and 7% of female ER-positive IDC. However, the expression pattern of NKX3.1 has never been previously studied in male breast cancer.

Design: Two tissue microarrays (TMAs) were constructed containing 28 cases of primary male breast carcinomas, consisting of 27 invasive ductal carcinomas (IDC) and 1 pure ductal carcinoma *in situ* (DCIS) with known ER, progesterone receptor (PR) and Her2 status. Clinicopathologic characteristics were recorded. TMAs were labeled by immunohistochemistry for NKX3.1 and AR. Any nuclear NKX3.1 labeling was considered positive. Nuclear AR labeling >1% was considered positive.

Results: The 28 patients ranged in age from 37-82 years, and 8 (29%) patients also had prostate carcinoma, either before or after the diagnosis of IDC. All male IDC were ER and PR positive, and 3 were Her2 positive. AR labeling was seen in 100% cases, 96% of which were strong and diffuse. Nuclear NKX3.1 labeling was seen in 7 (26%) male IDC and in the one case of DCIS; of the NKX3.1 positive IDC, 5 were Luminal A (ER/PR+, Her2-) and 2 were Luminal B (ER/PR+ Her2+). NKX3.1 labeling intensity was weak in all cases, with variable percentage expression (1-25%).

Conclusions: This is the first study to evaluate NKX3.1 in male breast carcinomas. NKX3.1 labeling is seen more often in male IDC (26%) than in female IDC (2%) (p-value = 0.0006). Even when controlling for phenotype, NKX3.1 labeling is seen more often in male ER-positive IDC (26%) than in female ER-positive IDC (7%) (p-value = 0.1415). These data suggest an altered role for NKX3.1 in male IDC as compared to female IDC. Furthermore, in the work-up of a metastatic carcinoma of unknown primary, the presence of NKX3.1 labeling in both prostate and breast carcinomas may cause confusion in patients with carcinomas of both origins, as seen in eight patients in this series.

124 Androgen Receptor Immunoexpression in Triple-Negative Breast Cancer

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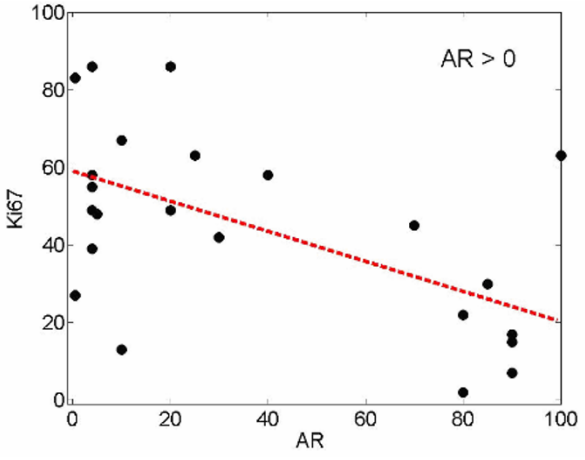
Background: Triple negative invasive breast carcinoma (TNBC) is a heterogenous subtype of breast cancer that represents a therapeutic challenge. Gene expression analyses have shown that androgen receptor (AR) is over expressed in a subset of TNBC and that these carcinomas may benefit from antiandrogen treatment. This study is aimed at determining the utility of AR expression as a prognostic and predictive marker in TNBC.

Design: 52 TNBCs diagnosed between 2010-2012 were retrieved from our database. Information including patients' age, sex, tumor size, lymph node status, treatment and follow up were collected. Patients ranged in age from 28 to 77 yrs. Breast carcinomas were fixed and evaluated in accordance with the 2007 ASCO/CAP guidelines. All patients received lumpectomy or radical mastectomy as primary treatment. Immunostains were evaluated by two pathologists. Concordant results were recorded. A cutoff value of 15% was used for EGFR positive cases while any positive staining was used as positive for AR. AR expression was correlated with tumor size, tumor grade,

lymph node status, EGFR, p53, Ki-67 expression and outcome. Statistical analysis was performed using a univariate Cox regression analysis, Chi-Square correlation test, Kaplan-Meier curves and log-rank tests.

Results: 25% (13/52) of TNBC expressed varying levels of AR. A statistically significant inverse correlation was observed between AR expression and proliferation rate as determined by Ki67 (p=0.003)(Figure 1). Higher levels of AR expression were associated with lower levels of Ki67 immunostaining. AR expression also correlated with EGFR expression (p=0.005). Positive staining was observed in 40% (8/20) of EGFR positive and 15.6% (5/32) of EGFR negative cancers. No statistically significant correlations were observed with tumor size , tumor grade, lymph nodes status or outcome.



Inverse correlation between AR expression and Ki67 expression.

Conclusions: Our study reveals that:

1. AR is expressed in a subset of TNBC.
2. AR expression correlates inversely with Ki67 in a linear fashion suggesting that AR positive TNBC may be less aggressive tumors.
3. AR immunostaining may be used for selecting TNBCs for targeted antiandrogen therapy.
4. Further studies are needed to confirm the predictive significance of AR.

125 A Laboratory Comparison of the 21-Gene Assay and PAM50-ROR

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Background: The 21-gene Recurrence Score® assay is validated in patients (pts) with ER+ early-stage invasive breast cancer (EBC) and predicts 10-year distant recurrence risk and chemotherapy (CT) benefit. The Prosigna® assay (ROR), which uses 46 of the PAM50 genes, was validated in post-menopausal pts with ER+ EBC and is a prognostic assay only. Despite differences in platforms and methods used for development and validation, it is frequently believed that the assay results are interchangeable. We performed a study comparing results from the two assays obtained from the same tumor blocks. The first 40 samples showed a substantial disagreement in how the assays stratify risk.

Design: 70 sequential BC tumors from Marin Medical Laboratories with sufficient tumor material were selected to be tested with the standard 21-gene assay. Samples were sent to an independent lab where Prosigna ROR and intrinsic subtype was performed with the operators blinded to Recurrence Score results. The first 40 cases were stratified by Recurrence Score (20 low, 10 intermediate and 10 high) Descriptive statistics were used to compare results from the 2 assays.

Results: Of the 40 initial pts evaluated, 7 were excluded: 3 for low RNA signal in the Prosigna assay and 4 were ER(-) by RT-PCR. Of the 33 remaining cases; 24 ductal, 7 lobular; 27 were N(-) and 6 were N+. The Spearman rank correlation between Recurrence Score and ROR was 0.40 (95% CI 0.06 – 0.65). Risk group assignment (low/intermediate/high) between Recurrence Score and ROR was in agreement in 56% (15/27) of N(-). Prosigna classified 19 luminal A, 12 luminal B, 2 HER2 enriched and 0 basal. In both the luminal A and B groups there was a wide range of Recurrence Score results.

Conclusions: Consistent with prior comparisons between the Onco*type* DX and other genomic assays, there are substantial differences in the way pts are risk stratified and it cannot be assumed that the assay results are interchangeable. These results suggest that there is only a modest agreement between Recurrence Score results and ROR, with almost half of N(-), ER+ pts classified differently, including ~30% of high ROR pts being classified as low risk by the Recurrence Score with expected minimal if any benefit from chemotherapy. Final data from 70 pts will be presented.

126 Role of TGF Beta Isoforms in Endocrine Positive/ Responsive Breast Cancer

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Background: The estrogen receptor (ER) pathway in breast cancer has been influenced by a volley of growth factors and inhibitors and hence the end results of endocrine therapy are variable.One of them is Transforming Growth Factor Beta (TGF-β). ERα blocks TGF-β pathway by multiple means, including direct interactions of its signaling components namely, Smads. This study addresses the impact of immunohistochemical (IHC) expression of TGF beta isoforms in prognosis of endocrine responsive breast cancer.

Design: Retrospectively, 150 patients with ER/PR/ both positive breast cancers who received tamoxifen treatment were studied for prevalence of ER beta and TGFβ 1, 2, 3 in a blinded fashion. After IHC was interpreted, the patients' clinical data was studied and impact of TGF beta isoforms on prognosis of patients was studied using Kaplan Meier analysis.

Antibodies to TGF beta isoforms used were :- Acris antibodies - Polyclonal Antibody to TGFb1 (AP 15830PU-N); Polyclonal Antibody to TGFb2 (AP15815PU-N); Polyclonal Antibody to TGFb3 (AP15833PU-N).

Results: The mean age of patients was 47.3 years and 78 patients were pre-menopausal, 60 post-menopausal and 10 peri-menopausal. Only 25(16.7%) were operable breast cancers, while 122(81.3%) had large operable or locally advanced breast cancers requiring neoadjuvant therapy. Ninety patients had axillary nodal metastases.

TGFb1/TGFb2/TGFb3 staining in epithelium of tumors was seen in 148(98.7%), 132 / 145 (91%), 106 / 142 (74.6%) tumors and in stroma in 35.5%, 18.6% and 9.1% tumors respectively. Tumors with TGFb3 staining were significantly low stage(p-0.048).

Factors impacting Disease Free Survival (DFS) favorably were ER beta (p-0.020), TGFb3 (p-0.013) while TGFb1 expression in stroma adversely impacted Overall Survival (OAS) (p–0.039). ER beta positive and ER beta negative groups were separately analyzed for OAS/DFS. In ER beta positive group, TGFb3 favorably(p- 0.003) impacted DFS while TGFb1 expression in stroma adversely impacted OAS(p-0.027). In ER beta negative patients, TGFb2 impacted OAS adversely.

Interestingly, in both ER beta negative (p-0.0001) and ER beta positive (p – 0.008) patients, coexpression of TGFb2 and TGFb3 was associated with better DFS as compared to tumors expressing TGFb2 only.

Conclusions: TGF beta isoforms have different and opposing action depending on the ER beta status and this could be exploited for future anti TGF beta therapy in endocrine positive breast cancer.

127 Significance of Epithelial Mesenchymal Transition (EMT) in Breast Cancer and Implications on Lymph Node Metastasis

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Background: Epithelial mesenchymal transition (EMT) originally described as an essential step for morphogenesis is now considered a major mechanism for the conversion of early stage tumours to invasive malignancies. In this study we analyzed the morphological transition of breast carcinoma cells from epithelial to mesenchymal phenotype and correlated with other histopathological and molecular parameters to evaluate its prognostic significance.

Design: Transition of human breast carcinoma cells from epithelial to mesenchymal phenotype was assessed in the pure epithelial cell population isolated by laser capture microdissection. Phenotypic transition was assessed by immunohistochemistry (IHC) using epithelial (E-cadherin, CD44, CD44v6) and mesenchymal markers (Vimentin). Regulators of EMT (Twist and HOXB7) were evaluated by IHC and real time RT-PCR. The association of EMT with tumour angiogenesis (CD31), luminal (CK8/18) and basal (CK14, CK5/6) phenotype was assessed by IHC.

Results: EMT was demonstrated in 21% cases as positivity for vimentin and complete loss or less than score 3 expression of E-cadherin. There was downregulation of adhesion molecules (CD44 and CD44v6) in cases of EMT and upregulation of regulators of EMT (twist and HOXB7) at protein and mRNA level. Of these 71.4% cases were grade II tumours and 28.6% cases were grade III tumours. Majority(66.6%) cases with EMT showed expression of the luminal marker (CK 8/18) and only 7 cases (33.3%) cases were positive for basal markers (CK14 and CK5/6). Only Ten cases of EMT, had lymph node metastasis, of which six cases showed expression of vimentin and loss of E-cadherin and the remaining four cases did not show expression of vimentin but showed focal E-cadherin and CD44 expression in contrast to the primary tumours, thereby indicating evidence of Mesenchymal-epithelial transition in metastatic deposits. Significant positive correlation of EMT was noted with tumour size (>2cm; p=<.001), tumour grade (p=0.012), and tumour angiogenesis (p=.007). Negative correlation was noted with lymphovascular emboli and lymph node metastasis (p=.001). However no correlation was noted with molecular subtypes (p=.368).

Conclusions: This study highlights that EMT does occur in invasive breast cancer, and correlates with tumour size, grade and angiogenesis, however no positive association with lymph node metastasis and molecular phenotype was noted. Future work to assess role of EMT in distant spread of breast cancer by haematogenous route and follow up for recurrence is needed.

128 Characterization of Breast Cancers With Equivocal and Non-Classical HER2 FISH Results – A Multi-Institutional Study

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Background: The 2013 CAP/ASCO HER2 Testing Guidelines Update modified HER2 FISH categories such that some cases with monosomy, co-amplification/polysomy, low-level increased HER2 signals or heterogeneity now are considered amplified or equivocal. This study examines the frequency and clinico-pathologic characteristics of breast cancers with equivocal or "non-classical" HER2 FISH results.

Design: Breast cancers (2001 to 2014) with HER2 FISH results, HER2 IHC, ER, grade and age from 3 institutions were collected. HER2 FISH was interpreted using the updated recommendations. Amplified cases with non-classical results were grouped into the following categories: 1) monosomy (ratio $\geq$ 2.0, mean HER2/cell < 4.0); 2) co-amplified (ratio < 2.0, mean HER2/cell $\geq$ 6.0); 3) low amplified (ratio $\geq$ 2.0, mean HER2/cell 4.0-5.9). Heterogeneous cases with clustered HER2 positive cells were also included.

Results: Of 7977 cases, 3.71% were equivocal and 4.29 % had a "non-classical" HER2 amplified result; 1.32 % monosomy, 0.64 % co-amplified, 2.08 % low amplified, and 0.25% heterogeneous. These cancers had a high frequency of ER positive (79%), Nottingham grade 3 (54%) results [Table 1]. The highest percentage of grade 3 cancers (73.8%) and concurrent 3+ HER2 IHC (24%) was in the co-amplified group. The monosomy group had the highest percent grade 1 cancers (13%). Equivocal cases had the lowest percent 3+ HER2 IHC (6.5%), but otherwise shared similar characteristics with the non-classical amplified categories.

Category	Mean Age	Grade 3 %	Grade 1 %	HER2 2+ %	HER2 3+ %	ER + %
Equivocal	58	45.7	8.7	67.7	6.5	82.3
Monosomy	56	44.6	13.0	60.4	13.2	79.1
Co-amplified	58	73.8	4.8	62.0	24.0	80.8
Low amplified	56	43.5	8.4	68.1	10.1	80.3
Heterogeneous	61	63.2	5.3	N/A	N/A	72.2

Conclusions: Cases with non-classical HER2 amplification or equivocal results are typically ER positive, higher grade cancers. Co-amplified cases have the highest rates of aggressive characteristics. These results support the current classification scheme for HER2 FISH, with case-by-case correlation with additional clinical-pathologic factors when evaluating whether to offer HER2 targeted therapies.

129 Anomalous Expression of Cytokeratin and p63 in the Stroma of Phyllodes Tumor: A Comparison With Spindle Cell Metaplastic Carcinoma

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Background: The stromal expansion and overgrowth in phyllodes tumors may cause diagnostic confusion with spindle cell metaplastic carcinomas, especially on core biopsies. Immunohistochemical stain for cytokeratin and p63 has been applies to distinguish them. However, Chia et al recently reported focal patchy cytokeratin stains in as many as 28% of phyllodes tumors.

Design: Twenty one phyllodes tumors (12 benign, 1 borderline, 9 malignant) were identified in department file from 2005 to now. Four tumor blocks were selected from each case. Immunohistochemical stain for cytokeratin cocktail (including AE1/AE3 and Cam 5.2) and p63 were performed. In addition, 22 metaplastic carcinoma composed entirely or mostly of spidnle cells were identified in file from 1997 to now. Immunohistochemistry profile was available for 15 of the 22 cases. Patient's age, tumor size and lymph node status were abstracted from the pathology report.

Results: Cytokeratin cocktail and p63 were focally (5% of the tumor cells) expressed in the stroma of the 2 of 9 (22%) malignant phyllodes tumors in a scattered/patchy pattern, while the stroma of benign and borderline phyllodes tumors was not stained with these markers. On the other hand, cytokeratin was negative in the spindle cells of metaplastic carcinomas in 3 of 15 cases, very focally positive in another 3 cases, while p63 was negative in one case and very focally positive in another case. Clinically, phyllodes tumor was diagnosed in the youger age group (mean age 41, ranging from 13 to 83) with larger tumor size (mean size 6.6 cm, ranging from 1.5 cm to 14 cm), compared with spindle cell metaplastic carcinoma (mean age 62.7, ranging from 42 to 87; mean size 3.4 cm, ranging from 1.0 to 10 cm). Two cases of the malignant phyllodes tumor had lymph node sampled and they are negative, while 4 of the 19 metaplastic carcinoma cases with lymph node sampled had metastatic disease in the lymph node (s).

Conclusions: Anomalous expression of cytokeratin and p63 could be present in the stroma of malignant phyllodes tumor, whereas spindle cell metaplastic carcinoma occasionally could be cytokeratin negative or very focal, even though p63 negativity is rare. Caution should be exercised when one is relying on those markers to make differential diagnosis, especially on core biopsies. Other clinical/pathological informations may have reference value.

130 Impact of the 2013 ASCO/CAP Guideline Recommendations for HER2 Testing of Invasive Breast Carcinoma: A Focus on Tumors Assessed as Equivocal for HER2 Gene Amplification By FISH

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Background: HER2 testing is routinely performed on primary invasive breast cancers. An update to the 2007 ASCO-CAP HER2 testing guidelines includes changes to HER2 in situ hybridization (ISH) interpretation criteria. The current 'equivocal' category places emphasis on mean HER2 copy number and is now defined as a HER2 count between 4.0 and <6.0, and a HER2:CEP17 ratio of <2.0, with additional testing recommended for such cases. We conducted a single center retrospective review of a consecutive cohort of primary breast carcinomas with available HER2 results to assess the impact of updated guidelines on HER2 classification, laboratory resources, and to better understand the pathobiology of tumors that are equivocal for HER2.

Design: FISH records were reviewed for all primary breast cancers that underwent dual-probe HER2 FISH testing from 2007-2013. We classified each case using both 2007 and 2013 guidelines and identified a subset that were initially reported as HER2 negative but are deemed equivocal by 2013 guidelines. Traditional pathologic factors of this subset were compared to HER2 negative (n=311) and positive (n=144) control cases.

Results: A total of 904 HER2 FISH tests were completed, most of which were performed for equivocal HER2 immunohistochemistry. 90.6% (819 cases) had no change in HER2 classification. Of the 85 (9.4%) cases with a classification change, 66 (7.3%) went from HER2 negative to equivocal, 15 cases (1.7%) were reclassified as HER2 positive, and 4 cases from HER2 equivocal to negative. Tumors reclassified from HER2 negative to equivocal were predominantly ER positive (90%). The three HER2 groups were significantly different with respect to tumor size ($p<0.0001$), Nottingham grade ($p<0.0001$), lymphatic/vascular invasion ($p<0.0001$), and lymph node status ($p=0.0063$). The differences were driven by comparisons between HER2 positive and negative control groups, with the study group showing intermediate features between both control groups.

Conclusions: The updated HER2 testing guidelines will result in the reclassification of approximately 9.5% of primary breast cancers with uncertainty regarding the clinical impact of this reclassification in the majority of cases. Resource utilization will increase as a result of the recommendation for retesting. Further work is needed to better elucidate the clinical-pathologic behavior of this novel group of HER2 'equivocal' primary breast cancers.

131 Genomic Alterations in Inflammatory Breast Cancer

Laura Biederman, Laura Austin, Massimo Cristofanilli, Juan Palazzo. Thomas Jefferson University Hospital, Philadelphia, PA.

Background: Inflammatory breast cancer (IBC) is an aggressive subtype that is resistant to standard chemotherapy. The aim of this study is to characterize the genomic alterations of IBC by studying circulating tumor DNA and next generation sequencing (NGS). This analysis can be used to identify pathways that may be specific for IBC as well as more effective targets for therapy.

Design: The study included 25 patients with IBC as defined clinically and pathologically. Data was collected from targeted NGS by Foundation One™ and circulating tumor DNA by Guardant Health. The data was analyzed for gene mutation type and cell-signaling pathway, disease subtype (ER/PR and HER2 status), and concordance rate between the two methods. Approval for the study was obtained from the IRB.

Results: The tumors included a diverse immunohistochemical profile (ER and PR positive, HER 2 positive only, and triple negative). Of the 25 patients analyzed, 84% (n=21) had a mutation in the p53 pathway (p53 or MDM) detected by either circulating DNA or NGS. There was a 47.6% concordance rate between both methods in p53 mutations. In addition, the number of p53 mutations remained above the expected average despite the immunohistochemical phenotype of the patient. There was also a mutation in the mTOR pathway (AKT, IRS, PTEN, or PI3K) in 60% (n=15), and a mutation in HER family (EGFR or ERBB2) in 56% (n=14) of the total number of cases analyzed. Of the patients who had a mutation in the EGFR pathway, 69% of them had HER2 positivity by immunohistochemistry. There does not seem to be a predominant pathway that emerges according to standard immunophenotypic profiles (ER, PR, and HER2). All the cases that expressed HER2 had an EGFR mutation, but a third of the cases with an EGFR mutation were negative for HER2 by immunohistochemistry.

Conclusions: This analysis shows IBC to be a genetically heterogeneous group of cancers. However, there was a high incidence of p53 mutation compared with non-IBC, underscoring its aggressive biology. A significant percent of cases had a mutation in either the mTOR (AKT, IRS, PTEN, or PI3K) or the EGFR (EGFR or ERBB2) pathways that could potentially be used for targeted therapy. Also noted, the method used in the analysis of genomic alterations may influence its detection rate, something that deserves more prospective evaluation.

132 Sentinel Lymph Node Evaluation for Metastatic Breast Carcinoma: The Impact of the ACOSOG Z0011 Trial

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Background: Results of the ACOSOG Z0011 trial showed that patients with T1-T2 breast cancer (BC) with 1 to 2 positive sentinel lymph nodes (SLN) treated with breast conservation and systemic therapy did not benefit from axillary lymph node dissection (ALND). Subsequently, most pathology departments have likely seen a decrease in SLNs sent for frozen section diagnosis. The purpose of this study is to determine the effect of the Z0011 trial on pathology practice and management of the axilla.

Design: The surgical pathology archives were searched for consecutive cases of primary BC treated with lumpectomy and SLN, where SLNs were sent for permanent section,

from 2010 to 2014. The histologic type, number of lymph nodes positive, the size of the largest metastatic deposit and whether or not ALND was performed were recorded.
Results: A total of 124 cases were identified. Twenty-one cases (21/124; 17%) had positive SLNs. Three cases (3/124; 2%) with positive SLN went on to ALND. Of these 3 cases, 2 cases had 3 SLNs with macrometastasis and the third case had 1 of 2 SLNs with a 3.0 cm deposit and extranodal extension. The remaining cases with positive SLNs had a final N stage of pN0(i+)(sn) (2 cases with isolated tumor cells), pN1mi(sn) (8 cases with micrometastasis) and pN1a(sn) with only 1 or 2 lymph nodes with macrometastasis (7 cases).
Conclusions: In the post Z0011 era, the number of SLNs sent for permanent section diagnosis has increased at our institution which has inadvertently saved the healthcare system the extra charges related to frozen section diagnosis. In our analysis of SLN evaluation in this setting, a very low percentage of cases (2%) went on to ALND. These results are likely a combination of adequate clinical staging of the axilla prior to surgery and criteria set by the Z0011 trial. Overall, our data provides evidence that frozen section evaluation of SLNs for metastatic BC should only be reserved for cases that do not meet criteria of the Z0011 trial.

133 Increased CD68-Positive Macrophages and CD4-Positive Lymphocytes in Tumor Associated Inflammation in Pregnancy Associated Breast Cancer May Contribute To a Poor Prognosis

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Background: Pregnancy associated breast cancer (PABC), diagnosed during gestation to 5 years post-partum, is associated with a poor prognosis. Those diagnosed within 2 years have an even worse outcome. The microenviroment may be tumor promoting after pregnancy as the mammary gland is remodeled to its pre-pregnant state. In this pro-inflammatory state, CD68+ macrophages promote invasive tumor cell growth and metastases, while CD4+ lymphocytes in breast carcinomas produce high levels of IL-4 which regulate the protumor activities of macrophages. We previously assessed the presence and degree of tumor associated inflammation (TAI) and reported that TAI was more prominent in PABC. In this study, our goal was to further characterize the components of the TAI in PABC.
Design: 38 patients diagnosed with PABC within 2 years of pregnancy (mean age=35.5, range=25-48) and control age-/stage-matched nulliparous women (mean age=37.5, range=29-48) were evaluated. Slides were reviewed and pathologic tumor characteristics and TAI were noted. Immunohistochemical stains for CD4, CD8, CD68 and CD137 were performed on 20 PABC and 15 control cases. Extent (1=1-25% positive tumor cells, 2=26-50%, 3=51-75% or 4=76-100%) and intensity (1=weak, 2=moderate or 3=strong) of staining were assessed. A composite score (CS) was calculated by multiplying the extent by intensity.
Results: PABC were more likely than controls to have more CD68+ macrophages (mean CS=10.2 vs. 5.8, $p<0.0001$) and CD4+ lymphocytes (mean CS=10.5 vs. 8.2, $p=0.016$). 80% of PABC cases had strong immunoreactivity for CD68 (CS=9-12), while only 27% of controls did ($p=0.0024$). Of interest, among the PABC, 83.3% with the strongest CD68 expression (CS=12) had positive lymph nodes compared to only 25% in those with CS<12 ($p=0.019$). Also, CD8+ lymphocytes (mean CS=9.8 vs. 7.8, $p=0.05$) and CD137+ lymphocytes (mean CS=4.0 vs. 2.4, $p=0.056$) were increased in PABC compared to controls.
Conclusions: 1. The majority of PABC have TAI. **2.** Increased CD68+ macrophages are present in PABC. **3.** PABC with high expression of CD68 have positive lymph nodes. **4.** PABC has more CD4+, CD8+ and CD137+ lymphocytes than controls. Our findings support the role of immunosurveillance in regulating metastatic spread and suggest that TAI, particularly enriched for CD68+ macrophages and CD4+ lymphocytes, may play an important role in tumor progression and metastasis in PABC and contribute to the poor prognosis in these aggressive breast carcinomas.

134 Novel B-250 Antibody Identifies Breast Cancers Expressing Progesterone Receptor A Isoform Only

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Background: Estrogen receptor (ER) and progesterone receptor (PR) play an essential role in breast tumor development, progression, and treatment. PR exists in two isoforms, PRA and PRB. Variable PRA/PRB ratios are reported in human breast tumors. The current gold standard PR antibody (clone 1294) does not differentiate between the two isoforms. In this study, we compare 1294 to other commercially available antibodies and the novel clone B-250.
Design: Tissue microarray (TMA) of formalin-fixed paraffin embedded (FFPE) cells expressing either PRA or PRB, and a human breast TMA (HBT) composed of 42 breast cancers and 12 normal breast tissues were created. Antibody clones 10A9, PGR-312, 1294, A0098, hPRa7, and B-250 (in-house) were optimized for immunohistochemical (IHC) use. 10A9 and PGR-312 have been claimed to recognize PRA only; and 1294, A0098, and hPRa7 to recognize both PRA and PRB. By Western blot, B-250 recognizes PRB only. Using the Allred score for IHC analysis, antibodies were compared to the clinically validated 1294 on the HBT. We tabulated the number of discrepant cases and calculated the degree of discrepancy (difference in Allred score). Significant discrepancy is defined as: 1) positive versus negative scores, or 2) a degree of discrepancy of 3 or more. Four human breast cancers consisting of 3 PRA/PRB-expressing and 1 PRA only-expressing tumors by Western blot were blindly stained with 1294 and B-250.
Results: In the cell line TMA, antibody clones 10A9, PGR-312, 1294, A0098, and hPRa7 recognized both PRA and PRB isoforms. Clone B-250 recognized only the PRB isoform. On the HBT, clones 10A9, PGR-312, A0098, and B-250 had a change between positive and negative when compared to 1294 in 6, 1, 3, and 2 cases, respectively. Significant degrees of discrepancy were observed in 7, 1, 7, and 3 cases, respectively. Clone hPRa7 was negative in all human tissue analyzed. IHC staining of clones 1294 and B-250 correctly identified the PRA only expressing human breast cancer.
Conclusions: In contrast to previously published reports, our data show that clones 10A9 and PGR-312 are not specific for PRA. PGR-312 and 1294 showed similar staining patterns in HBT. We presented the novel antibody B-250 that recognizes only PRB by IHC. We reported for the first time the ability to identify PRA-only expressing human breast cancers by immunohistochemistry. As PRA confers a more aggressive behavior, an IHC assay for detection of PR isoforms in breast cancer specimens is of potential clinical value.

135 Monosomy of Chromosome 17 in Breast Carcinoma During Interpretation of Her-2/neu Gene Amplification

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Background: Recent guidelines (ASCO/CAP 2013) addressed and get guide to appropiate Her-2/neu test scoring. Among uncommon patterns of Her-2 abnormalities, monosomy of chromosome 17 may affect the interpretation of Her-2/neu amplification. Its prevalence ranges from 1% up to 38% of breast carcinoma. We sought to evaluate the impact of monosomy of chromosome 17 to interpretation of Her-2/neu gene status.
Design: 201 breast carcinoma were reviewed at genomic level for Her-2/neu gene amplification. FISH analysis was performed by using double probes (LSI/CEP) (Abbott). Absolute gene copy number was scored per each probe. Her-2 FISH test was repeated on serial tissue sections, ranging in thickness from 3 to 20 µm. Ratio was initially scored and subsequently corrected by monosomy after control tests using the aCGH method to overcome false interpretation due to nuclear truncation. Her-2 immunotests (Hercept Test) was performed on all cases.
Results: 26/201 cases were amplified (13%). Single signals per CEP17 were revealed in 7/201 (3,5%) cases. Five out of 7 cases appeared monosomic after matching with aCGH analysis and evidenced single signals in >60% of nuclei after second-look on FISH analysis. Among 5, one case showed amplification with a pattern 7/1 (Her-2/CEP17>2) of copies (3+ at immunotest); three cases revealed single signals per both probes (LSI/CEP=1) and one case revealed a 3:1 (LSI/CEP>2) count ratio; importantly all last 4 cases showed 0/1+ score at immunotest.
Conclusions: 1) monosomy of chromosome 17 may be observed in 3,5% of breast carcinoma; 2) the skewing of the ratio due to single centromeric 17 probe may bring to false positive evaluation of gene amplification; 3) monosomy due to biological reasons rather than nuclear truncation was observed when cut-off set to 60% of nuclei; 4) when dealing with cases showing a 3:1 ratio (Her-2/CEP) the interpretation should be “Her-2 negative”.

136 Optimizing Cytology Cell Blocks for More Cellular and Reliable Immunohistochemical Results in Breast Cancer Specimens

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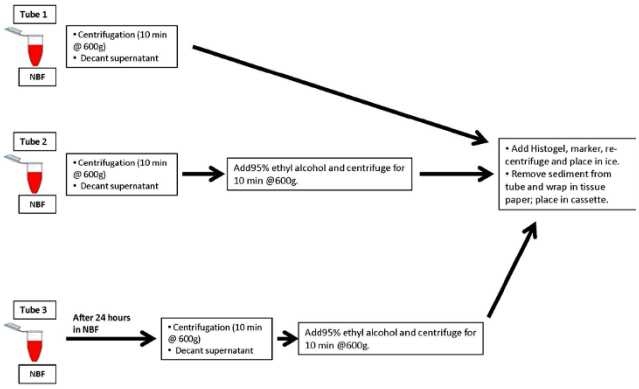
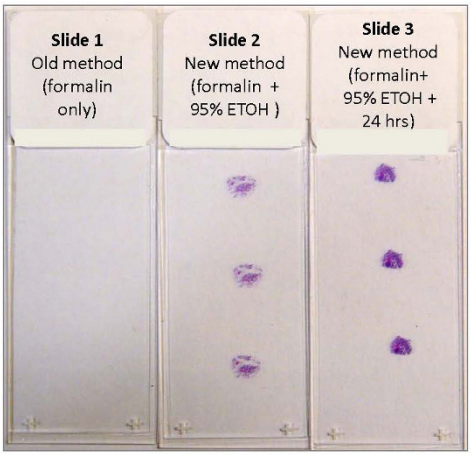
Background: Cell blocks (CB) with adequate cellularity are important for diagnosis and ancillary studies. Fine needle aspiration is often used for diagnosing metastases and lesions that are not amenable to core biopsy.
Cytology samples from breast cancer, primary or metastatic, are unique in that they must be fixed in 10% neutral buffered formalin (NBF) to comply with the ASCO/CAP guidelines for evaluation of estrogen and progesterone receptors (ER, PR) and HER2 by immunohistochemistry (IHC).
The aim of this study was to determine whether the addition of 95% ethyl alcohol (ETOH) into NBF and an additional centrifugation step would improve cellularity of CB without compromising ER/PR/HER2 IHC.
Design: Eighteen breast cancer specimens, >1 cm in size and known ER+ and/or HER2(3+) status were selected. Scrapings from each case were performed at the time of grossing and placed in 50ml conical tubes all with NBF labeled 1, 2 and 3. Processing for Tube 1 followed prior established CB method (old method) and for Tubes 2 and 3, the newly proposed CB method (new method) as seen in Figure 1. Histological processing followed standard cycle for CB with adequate fixation time. CB were evaluated for cellularity, stained for ER and HER2, and scored.

Figure 1



Results: The CB with the ETOH + centrifugation step (Tubes 2, 3) showed better cell aggregation (see example in Figure 2). 17/18 CB (94%) had similar or improved cellularity. There was a 93% concordance of ER regardless of CB processing method (same for all tubes) and 100% concordance of HER2 between CB and the paired biopsy/resection.



Conclusions: Adding ETOH + centrifugation step into the CB processing provides the same or better cellularity in the majority of cases while complying with the ASCO/CAP guidelines. Better cellular aggregation in the slide helps screening, IHC analysis and possibly molecular testing. Staining pattern of ER and HER2 IHC in the newly proposed CB preparation method is reliable.

137 Targeted Next Generation Sequencing of Phyllodes Tumors Identifies Frequent MED12 Mutations and Opportunities for Targeted Therapy

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Background: Phyllodes tumors (PT) are fibroepithelial tumors with variable clinical behavior accounting for ~1% of all breast neoplasms. They are categorized histopathologically as benign, borderline and malignant, yet this classification is imprecise in predicting clinical behavior. In addition, efficacious treatment options for the 10% of all phyllodes tumors that progress to metastatic disease are lacking and survival rates are dismal. Despite the need for better molecular characterization and individualized treatment options, little is known about driving genetic alterations in PT.
Design: We used targeted, PCR based next generation sequencing (NGS) using a novel pan-cancer informed panel (the Oncomine Cancer Panel) on the Ion Torrent sequencing platform to identify somatic alterations in 15 formalin fixed paraffin embedded samples from 5 cases each of malignant, borderline and benign PT.
Results: NGS revealed hotspot mutations in *MED12* in 10/15 (67%) cases spanning all three histological grades in the known exon 2 hotspot. Mutations were similar to those recently observed in uterine leiomyomas and breast fibroadenomas. *TP53* mutations were identified exclusively in malignant tumors (3/5) and additional deleterious mutations in the tumor suppressors *RB1* and *NF1* were similarly exclusive to malignant cases. High level copy number alterations were nearly exclusively confined to malignant tumors, including potentially clinically actionable amplifications in *IGF1R* (2/5) and *EGFR* (1/5) in malignant cases.
Conclusions: Taken together, this study informs on the genetic alterations underlying phyllodes tumor development, suggests potential molecular correlates to histologic grade, expands the spectrum of tumors with frequent *MED12* hotspot mutations, and identifies *IGF1R* and *EGFR* as potential therapeutic targets in malignant cases.

138 Percent of Breast Cancers Positive for HER2 Varies By Ethnicity and Social Determinants of Health in California – Implications of Patient Demographics on Laboratory Benchmarks

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Background: The percentage of HER2 positive breast cancers by geographic region in California was previously shown to vary by the stage and age at presentation. It is unknown how additional population characteristics such as ethnicity, socioeconomic status, and insurance type also influence these rates. Our goal is to improve predictive models for expected laboratory HER2 positive rates in breast cancers by examining the impact of these additional demographic factors.
Design: Utilizing data from 2006-2011 from the California Cancer Registry (CCR), we examined the HER2 status of breast cancers as well as race/ethnicity, neighborhood quintile of socioeconomic status (SES), insurance type, and patient SES distribution by hospital in 29 NCI-modified health service areas in California.
Results: 121,408 out of 138,563 total (87.6%) breast cancers had available HER2 status. The highest percentage of HER2 positivity was seen in breast cancers from the Hispanic (20.15%), Chinese (21.93%), Filipina (24.79%), and other Asian/PI (22.34%) patient populations with the lowest in non-Hispanic whites (14.48%) and Japanese (14.06%). Patients receiving care at NCI cancer centers had a higher percent of HER2 positive cancers (18.05%) than those at non-NCI centers (16.50%). When grouped by insurance type, the highest percent HER2 positive cancers where in the not insured/self-pay (21.20%), public/medicaid (19.49%), and military (21.55%) groups compared with private insurance (16.64% HER2 positive). Reporting hospitals with over half of patients in the highest SES category had a lower percent of HER2 positive cancers (14.96%) compared to hospitals with the majority in the lowest SES (20.21%). Overall, the percent of HER2 positive cases decreased with increasing socioeconomic status as follows: 20.02% for 1st quintile, 17.97% for 2nd quintile, 16.62% for 3rd quintile, 15.99% for 4th quintile, and 14.69% for 5th quintile. Some geographic variation still existed when controlling for SES within each geographic region.
Conclusions: In California, the percent of breast cancers that are HER2 positive varies by ethnicity and SES. We plan to create a multivariate model to predict a laboratory's expected percent HER2 positive breast cancers which includes weighting for patient demographics. This information can be useful when setting laboratory benchmarks and can highlight possible issues in HER2 test performance.

139 Estrogen and Progesterone Receptors Are Expressed in Non-Neoplastic Breast Stroma

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Background: According to the ASCO/CAP guidelines for immunohistochemical testing of estrogen receptor (ER) and progesterone receptor (PR) in breast cancer [Arch Pathol Lab Med. 2010;124:e48-72], "The normal breast tissue also represents a useful built-in negative control of the staining because the myoepithelial cells and the stromal cells must invariably show a negative result." However, mammary myofibroblastomas--proliferations of the mammary stromal myofibroblasts--are known to have positive labeling for ER and PR, suggesting that the benign myofibroblasts would also label for nuclear hormone receptors. Here, we systematically evaluate ER and PR labeling of non-neoplastic breast stroma.
Design: 100 consecutive cases of core needle biopsies (CNB) with ER and PR immunohistochemistry (IHC) were retrieved from the pathologic archives. Clinicopathologic characteristics including age, race, diagnosis, tumor grade, and tumor ER status were documented. Exclusion criteria included slides of ER-negative tumors lacking benign breast lobules as internal controls, and diffuse infiltrating lobular carcinoma that might confound interpretation of benign stromal cell ER/PR labeling. ER and PR labeling in the benign breast stromal cells adjacent to ("tumor-associated stroma," i.e., within two high power fields) and stromal cells separate from ("non-tumor stroma") any carcinoma were recorded by documenting the intensity and distribution of nuclear labeling.
Results: 89 cases were included for review. 77% CNB contained invasive carcinoma, with 22% containing *in situ* carcinoma, and the majority (85%) of carcinomas were ER positive. 75% CNB contained both tumor-associated and non-tumor stroma, with the remaining 25% containing only tumor-associated stroma. Any stromal ER labeling was seen in 83% of CNB, including in the stroma associated with 92% of ER-negative carcinomas. Stromal ER labeling was seen in 89% tumor-associated stroma and in 83% of non-tumor stroma (p=0.33). Stromal PR labeling was seen in 15% of CNB, all of which also contained ER positive stromal cells. There was no statistical association between ER or PR stromal labeling with patient race, carcinoma type, carcinoma grade or carcinoma ER status. Myoepithelial cells within the benign lobules in the majority of cases were negative for ER and PR.
Conclusions: Benign mammary stromal myofibroblasts do label for nuclear ER and PR, as would be expected given the positive labeling seen in neoplastic myofibroblastomas. The use of mammary stromal cells as negative controls for ER and PR IHC is not recommended, however myoepithelial cells are overwhelmingly negative.

140 Measurement of Domain Specific HER2 (ERBB2) Expression May Classify Benefit From Trastuzumab in Breast Cancer

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Background: The ASCO/CAP guidelines consider chromogen-based immunohistochemistry (IHC) as the primary assay to determine HER2 status in breast cancer. Studies have shown that antibodies targeting the intracellular [ICD] or extracellular domains [ECD]) of HER2 are equivalent when the chromogenic method is used. Here we differentially quantified HER2 ICD and ECD expression, and assessed the prognostic value of domain specific HER2 results in patients who received adjuvant Trastuzumab therapy.

Design: We measured HER2 protein expression with quantitative immunofluorescence (QIF) and IHC in a HER2 standardization tissue microarray (TMA) using previously shown ICD and ECD antibody assays. Population-based cut-points were generated using Joinpoint software without considering outcome. HER2 IHC and FISH results were used as reference to determine sensitivity and specificity of the domain specific results. We assessed the prognostic value of ICD and ECD expression in 180 patients from a clinical trial of adjuvant chemotherapy followed by Trastuzumab (HeCOG 10/05).

Results: : HER2 ICD and ECD expression by QIF showed similar sensitivity and higher specificity to predict *HER2* gene amplification than IHC. In the HeCOG 10/05 trial specimens. 15% of cases showed discordant results for ICD and ECD expression. High ECD was significantly associated with longer disease-free survival (DFS) (log-rank *P*=0.049, HR=0.31, 95% CI: 0.144-0.9970.144-0.997), while ICD status was not. Among patients with low ECD, there was no difference in DFS by ICD status. However, when ICD was high, high ECD was significantly associated with longer DFS (log-rank *P*=0.027, HR=0.23, 95% CI: 0.037-0.82) compared to low ECD.Neither ICD nor ECD showed prognostic value in the 462 patients that were traditionally classified as HER2 negative and did not receive trastuzumab.

Conclusions: Performance to predict *HER2* amplification is affected by domain and detection technique. Quantitative measurements of HER2 ICD and ECD expression in breast cancer suggest a subclassification of HER2 positive tumors. Trastuzumab-treated patients with high ECD have better DFS than patients with low ECD. Elevated ICD alone has no prognostic value. This observation raises the possibility of differential benefit from trastuzumab therapy based on HER2 ECD expression.

141 Prolactin Levels, Expression of Downstream Signals in the Prolactin Receptor Pathway and Breast Cancer Risk: A Nested Case-Control Study From the Nurses' Health Study

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Background: Prolactin is a potent mitogen for the development of breast carcinoma through its transmembrane receptor (PRLR) and downstream signaling pathways: PI3K/MEK/AKT and JAK2/STAT5. Prolactin, when bound to PRLR, activates the JAK kinases resulting in STAT5 phosphorylation (pSTAT5) localizing it to the nucleus. pSTAT5 modulates expression of key target genes involved in growth, differentiation and survival and has been shown to have a role in resistance to antiestrogen therapy. While circulating prolactin has been related to breast cancer risk, it is not known whether this varies by tumor expression of proteins in the signaling pathway.

Design: We used conditional logistic regression to examine the association between prediagnostic circulating prolactin levels (>vs< median of 11 ng/mL) and breast cancer risk by tumor expression of markers in the PRLR pathway in a case-control study nested within the Nurses' Health Study. The analysis included 787 women with breast cancer and 1226 controls matched on age, fasting status and timing of blood collection. PRLR, pSTAT5 and pJAK2 expression was evaluated on immunostained breast cancer TMAs. For PRLR and pSTAT5, cores were scored from 0-3+ for cytoplasmic(c) and nuclear(n) staining intensity; a case was considered + if $\geq$1 core was scored ^{3}2+ except c-pSTAT5 which was scored + if any core was ^{3}1+ due to small numbers of cores scored ^{3}2+. pJAK2 was scored + if >1% of nuclei expressed the biomarker.

Results: The % positive cases are: c-PRLR=23%, n-PRLR=26%, c-pSTAT5=74%, n-pSTAT5=25% and pJAK2=81%. Women with higher plasma prolactin levels had a 2-fold increase in risk of n-pSTAT5+ tumors compared to women with lower levels (OR=2.0, 95%CI:1.3-2.9). However, there was no association between prolactin levels and n-pSTAT5 negative tumors (OR=1.05, 95%CI:0.8-1.3). There were no differences in the association between prolactin levels and breast cancer risk by PRLR, c-pSTAT5 or pJAK2 expression.

Conclusions: Our findings suggest elevated plasma prolactin levels may preferentially increase risk of breast tumors with high pSTAT5 expression. Confirmation of these results is needed, as well as an evaluation of whether women with elevated prolactin levels and pSTAT5+ tumors may show less resistance to antiestrogen therapy than those with pSTAT5 negative tumors (Peck, JCO 2011).

142 FGFR1 and EGFR Amplification in Breast Cancer

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Background: Breast cancer is the second leading cause of cancer related death in women. Recent advances allowed the identification of common targetable genomic alterations. Fibroblast growth factor receptor 1 (*FGFR1*) and epidermal growth factor receptor (*EGFR*) have been implicated as therapeutic targets in various cancer types. However, copy number aberrations in these genes remain under-characterized in breast cancer. Here, we explore *FGFR1* and *EGFR* amplification in breast carcinomas using molecular inversion probe array (MIP).

Design: We selected 43 invasive adenocarcinoma of breast from the archives of MD Anderson Cancer Center (2011-2014). Based on hormonal receptor (HR) expression by immunohistochemistry and *HER2* amplification by fluorescence in situ hybridization, the cases were classified into 4 groups: HR+/HER2- (n=15), HR+/HER2+ (n=14), HR-/HER2+ (n=8), and HR-/HER2- (n=6). After manual micro-dissection of tumors from formalin-fixed, paraffin-embedded tissue (FFPE), genomic DNA was extracted and subjected to MIP by OncoScan FFPE Assay kit and OncoScan Console software (Affymetrix, Santa Clara, CA). Copy number analysis of *FGFR1* and *EGFR* was performed by Nexus Express for OncoScan (BioDiscovery, El Segundo, CA). We defined gene amplification with a designated cut-off of 4.0 for high copy number gains. Four selected cases were also analyzed for *FGFR1* amplification by next generation sequencing (NGS) using Ion Torrent PGM and 50 gene hotspot panel (LifeTechnologies, Carlsbad, CA).

Results: Ten of 43 cases (23%) showed *FGFR1* amplification (copy number: 4.0-22) and 11 of 43 cases (26%) displayed *EGFR* amplification (copy number: 4-7). The results are summarized in Table 1. Interestingly, we observed co-amplification of *FGFR1* and *EGFR* only in HER2+ cases (6/22) but not in HER2- cases (0/21; p=0.02). In the 4 cases with *FGFR1* amplification but without *EGFR* amplification, *FGFR1* and *EGFR* copy number status was also confirmed by NGS analysis.

Conclusions: In our HER2+ cohort, there is correlation between *FGFR1* amplification and *EGFR* amplification. In contrast, no co-amplification of *FGFR1* and *EGFR* is detected in HER2- cases. These findings suggest that breast tumors can be further subtyped with different implications for targeted therapy.

HR/HER2	FGFR1+/ EGFR+	FGFR1+/ EGFR-	FGFR1-/ EGFR+	FGFR1-/ EGFR-	Total
+/-	0	3	4	8	15
+/+	4	0	0	10	14
-/+	2	0	0	6	8
-/-	0	1	1	4	6
Total	6	4	5	28	43

143 Are We Ready for Implementation of Bright-Field HER2 Dual In Situ Hybridization (DISH) Assay in Routine HER2 Testing?

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Background: Assessment of HER2 status by fluorescent hybridization in situ hybridization (FISH) or IHC has been standard of practice. Recently, DISH assay was approved by the FDA for HER2 testing. Although both *in-situ* methods (FISH and DISH) are performed on paraffin tissue, DISH utilizes light microscopy in assessment of HER2/CEP17 signals. While comparative data on DISH with other tests (FISH and IHC) have been published; the experience of DISH assay as a routine test has not been widely published. The aim of this study is to evaluate the performance of DISH in our laboratory as a routine test.

Design: Dual ISH staining results in visualization of HER2 as discrete black signals and CEP17 as red signals in normal (serving as internal positive controls) as well as in tumor nuclei using standard light microscopy. Four (4) -mm tissue specimen sections were used on the Benchmark XT staining platform (Ventana Medical Systems, Tucson, AZ). Statistical analysis was performed with calculation of *P* value using Graph Pad Prism 6 software.

Results: From January 2013 – August 2014, the DISH assay was performed on 490 breast cancer tissues (including 30 cases for initial validation). Five (5) cases were excluded due to absence of microinvasive carcinoma. 11% (49/452) were amplified; 2% (7/452) showed borderline amplification; 87% (396/452) were non-amplified (NA) Among the NA cases, 2% (6/396) showed aneusomy. In a subset of cases (validated and heterogeneous cases reflexed to FISH) the comparison of DISH to FISH ratio showed a high concordance rate (*P value = 0.2451*}; although DISH showed lower HER2 copy numbers. Technical difficulties were encountered in 3% (13/452) cases and were repeated and reflexed to an alternate test (FISH/IHC).

Conclusions: We report the experience of HER2 DISH assay which undeniably has additional advantages. Reflex testing to IHC/FISH is recommended in presence of heterogeneity of signals, staining issues as well as clusters. DISH appears to identify CEP17 anomalies (aneusomy), however this needs to be confirmed by additional FISH test. Caution should be applied in decalcified specimens as DISH fails in majority of cases. Identifying these disparities (with strict adherence to ASCO/CAP clinical practice guidelines) would assist in successful implementation in the routine workflow.

144 Intraoperative Touch Prep Evaluation of Sentinel Lymph Nodes in Breast Carcinoma

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Background: Axillary lymph node status is an independent prognostic indicator in breast cancer. Intraoperative identification of macrometastatic carcinoma in sentinel lymph nodes allows axillary dissection at the time of primary tumor excision. Intraoperative lymph node evaluation by touch prep analysis preserves all diagnostic tissue and maintains cytologic features for permanent section analysis.

Design: Consecutive cases of breast carcinoma surgical cases with sentinel lymph node evaluation were identified by pathology report search over a 2 year period. The clinical, radiologic and pathologic data was collected. Patients receiving neoadjuvant chemotherapy were excluded.

Results: Sentinel lymph nodes were excised in 414 patients. 516 touch preps were performed on 213 patients (Table 1).

	Intraoperative Evaluation (n=213)	No Intraoperative Evaluation (n=201)	PValue
Mean Age(years)	57.0	60.2	0.47
Surgeryperformed			
Mastectomy	171(80.3%)	58(28.9%)	
Lumpectomy	42(19.7%)	143(71.1%)	
TumorType			
DCIS	48(22.5%)	22(10.9%)	0.002
Invasive	165(77.5%)	179(89.1%)	0.002
CompletionDissection			
Same day	15(7%)	N/A	N/A
Second surgery	6 (2.8%)	10(5%)	0.25
PositiveLymph nodes			
Macrometastasis	24(11.3%)	22(10.9%)	0.92
Micrometastasis	10(4.7%)	5(2.5%)	0.23

Although lymph nodes were evaluated intraoperatively more frequently in patients undergoing mastectomy than those undergoing lumpectomy, there was no statistical difference in the rate of lymph node metastasis. Patients with intraoperative evaluation of lymph nodes underwent a second surgery for completion axillary dissection less frequently, but the difference was not statistically significant. "Positive" touch preps were seen in nodes with both micro- and macrometatases on final evaluation resulting in specificity, sensitivity, negative predictive and positive predictive values of 99, 60, 96 and 88% respectively for identifying macrometastases (Table 2).

	Permanent Result		
IntraoperativeResult	Negative	Micrometastasis	Macrometastasis
Negative(n=481)	460	5	16
Atypical(n=8)	6	2	0
Positive(n=27)	0	3	24

Conclusions: Touch prep analysis of lymph nodes showed low sensitivity for the detection of macrometastases, did not reduce the rates of re-operation and could not distinguish micro- from macrometastases.

145 Systematic Review of Clinical, Pathologic and Radiologic Parameters in Breast Atypia: Completeness of Lesion Excision at Biopsy and Atypia Subtype Are Predictive of Rate of Upgrade To Malignancy on Excision

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Background: Management of biopsy (Bx)-proven breast atypia is controversial due to difficulties in diagnostic reproducibility and fear of patient overtreatment in light of the low risk of underlying life-threatening malignancy. We aimed to design a predictive clinical nomogram for breast atypia, incorporating a 3-tiered grading system for atypia severity.

Design: We undertook review of all relevant histology & radiology of 227 patients with atypia on breast Bx and subsequent in-house excision (2008-2011). Bx lesions were assessed for 27 separate parameters (8 clinical, 10 radiologic, 9 pathologic) and univariate (UV) & multivariate (MV) statistical analyses performed. An atypia grading system (AGS) ("reactive/borderline atypical" [AGS1], "atypical NOS" [AGS2] and "atypical/borderline DCIS" [AGS3]) was designed and an inter-pathologist reproducibility study performed.

Results: 78% of patients had a Dx of ADH. 52% had either personal (PHx) or first-degree family history (FHx) of breast cancer. 45 patients (19%) had an upgrade to malignancy at excision; 35 had DCIS only. All 10 invasive tumors were T1N0. On UV analysis, PHx/FHx (OR 2.18,p =0.03), age (OR 1.45, p=0.04), atypia subtype (ADH vs FEA; OR 4.94, p=0.01), AGS (AGS1 vs AGs2: OR 0.32; AGS3 vs AGS2: OR 6.56, p<0.001), completeness of lesion removal at Bx (OR 2.54, p=0.008) and needle gauge (£11G vs >11G; OR 3.98, p=0.02) were statistically significant. A reproducibility study showed only moderate agreement between 4 pathologists for AGS (κ=0.4); thus AGS was not carried forward to MV analysis, where only 2 factors proved significant (completeness of excision and atypia subtype).

Conclusions: Of 27 parameters assessed, only completeness of lesion removal at Bx and atypia subtype showed a significant change in rate of malignant upgrade at excision. A novel AGS showed insufficient interobserver agreement to form the basis of a clinical nomogram. The predominant upgraded Dx at excision was DCIS. Variations in radiologic and pathologic practice mean that the design of a universally applicable nomogram for breast atypia management is extremely challenging. Multidisciplinary assessment of the individual risk/benefit ratio of excision in each case is likely to most benefit patients.

146 Effect of OncotypeDX Recurrence Score on Adjuvant Treatment Recommendations for 300 Early Stage, ER Positive Breast Cancer Patients in Ireland

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Background: Genomic analysis, utilizing tests such as OncotypeDX (Genomic Health), aids selection of patients for adjuvant chemotherapy. OncotypeDX reports a low, intermediate or high Recurrence Score (RS). The National Cancer Control Programme (NCCP) approved use of this test in all ER positive, early stage invasive breast cancer patients in Ireland in November 2011. This study investigated the impact of the RS on the decision to treat an Irish breast cancer cohort with adjuvant chemotherapy.

Design: 300 patients with node-negative, ER positive, Her2 negative invasive breast cancer with RS results from November 2011- January 2013 were identified from the pathology databases of two Irish hospitals. Clinicopathological details including age at diagnosis, tumor histology, size, grade and presence of lymphovascular invasion were collated. Treating oncologists were asked to review clinicopathological parameters and submit adjuvant chemotherapy decision with and without knowledge of RS. Adjuvant chemotherapy decisions pre and post OncotypeDX RS results were compared.

Results: 59.7% (n=179) of patients had a low RS, 40% (n=90) had intermediate RS and 10.3% (n=31) had high RS. Pre OncotypeDX testing, 74% (n=222) were considered potential candidates for adjuvant chemotherapy. With knowledge of the RS, 77.7% (n=233) of patient were not offered chemotherapy and 22.3% (n=67) were recommended to receive chemotherapy. A change in treatment decision was seen in 57% (n=171) (p value=0.000). The medical oncologist's decision to recommend chemotherapy and endocrine therapy was changed to endocrine therapy only in 54.3%(n=163). 70% of these patients had a 'low risk' RS. 2.7% (n=8) of patients originally considered for hormonal therapy alone were recommended chemotherapy with knowledge of RS.

Conclusions: RS had a significant impact on clinical practice. Compared with traditional clinicopathological assessment, incorporation of the RS result was associated with treatment recommendation changes for 57% of patients, with decision to omit chemotherapy in the majority of these patients. OncotypeDX RS results in an overall reduction in use of adjuvant chemotherapy.

147 MCT1 Expression in Breast Cancer: Evidence of Increased Mitochondrial Metabolism in Triple Negative Invasive Ductal Carcinomas

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Background: Monocarboxylate transporter 1 (MCT1) is a transporter of catabolites such as lactate and a marker of mitochondrial metabolism. Breast cancers with high MCT1 expression have a more aggressive behavior. Triple negative breast cancers (TNBC) are associated with poor outcomes and chemoresistance. The aim of this study was to evaluate the expression of MCT1 in a broad group of invasive ductal carcinomas (IDC). Digital pathology was employed for the interpretation of the stains.

Design: MCT1 expression was evaluated in IDC by immunohistochemistry on formalin-fixed paraffin-embedded tissue micro-arrays. Human tissue was obtained from the tumor bank of the Department of Pathology under a protocol approved by the IRB. Of the178 cases analyzed, 110 cases were estrogen receptor and/or progesterone receptor positive (ER+ and/or PR+), 48 were HER2+, and 55 were TNBC. MCT1 expression was quantified by digital pathology with Aperio software. The intensity of the staining was measured on a continuous 0 (black) to 255 (bright white) scale using a co-localization algorithm. Statistical analysis was performed using a linear mixed model.

Results: MCT1 expression was higher in TNBC compared to ER+ and/or PR+ with an average difference in intensity of 21.2 [17.9 - 24.5] ($p < 0.001$) and, it was also higher in TNBC compared to HER2+ with an average difference in intensity of 20.9 [16.8 - 25.1] ($p < 0.001$). We selected a cut-off value of 181.2 based on R.O.C., which revealed that 91% (50 out of 55) of TNBC had positive MCT1 staining compared to 29% (32 out of 110) of ER+ and/or PR+ and 31% (15 out of 48) of HER2+ IDC.

Conclusions: MCT1, which is a marker of high catabolite uptake and mitochondrial metabolism, is found highly expressed in TNBC compared with non-TNBC. The hypermetabolic status of the TNBC may contribute to their aggressive behavior and chemoresitance. MCT1 expression as detected with digital image analysis may be useful to design clinical trials using MCT1 inhibitors. A small subset of TNBC (9%) showed low expression of MCT1 demonstrating heterogeneity in the metabolic characteristic of these tumors.

148 A Feature Learning Framework for Reproducible Invasive Tumor Detection of Breast Cancer in Whole-Slide Images

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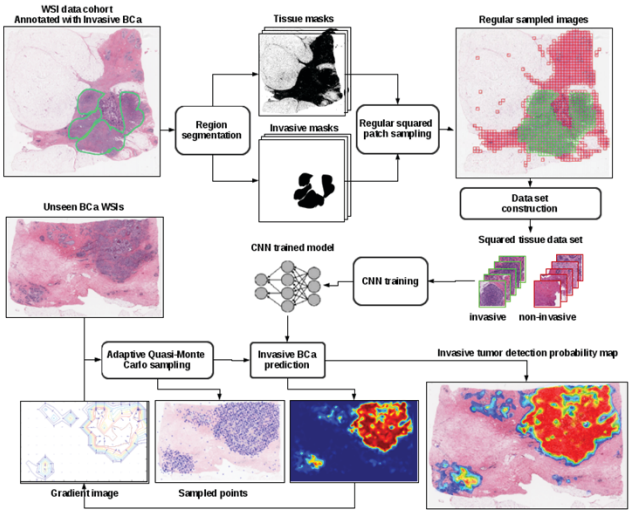
Background: Breast cancer (BCa) is the most common women cancer and the 2nd cause of cancer-related death in developed countries. Invasive breast cancers have worse prognosis than *in situ* types, with 90% of BCa cases. Isolation of invasive tumor is the first diagnostic step and allows further analysis via the well-known Nottingham grading scheme. Despite other methods to estimate the tumor grade, manual delineation from pathologists is still required and has significant inter-observer variability. In this work, we present an automated and reproducible feature learning framework to identify invasive tumor regions in whole-slide images (WSI).

Design: WSI, from an institutional data cohort with manual annotations of invasive regions from pathologists, were used to extract square tiles from invasive and non-invasive tissues. The most successful feature learning model for computer vision, convolutional neural network (CNN), is trained to predict the probability of invasive tumor tissues together with a high-throughput adaptive sampling. The reproducibility was evaluated by training two models from two different institutional data cohorts, University Hospitals Case Medical Center (UHCMC, n=110) and Hospital of the University of Pennsylvania (HUP, n=239), and validation from Cancer Institute of New Jersey (CINJ, n=40).

Results: The two trained CNN models, from HUP and UHCMC data cohorts, were evaluated into the CINJ validation data cohort in terms of area under ROC curve (AUC) in the square tile setup to classify between invasive and non-invasive tissues, obtaining AUC=0.9042 and AUC=0.8950 respectively. [Figure1] shows the complete feature learning framework and the final results into an unseen WSI.



Train cohort (cases)	Validation cohort (cases)	AUC
HUP (239)	CINJ (40)	0.9042
UHCMC (110)	CINJ (40)	0.8950

Figure 1: The training stage starts with the mask generation of tissue and invasive BCa regions in the WSI from a complete data cohort. These masks are used to identify the invasive and non-invasive tissue regions. Then a regular sampling is performed to generate a squared-tile based data set to train a CNN model for classification task to distinguish between invasive and non-invasive. The prediction stage for unseen WSI is performed with our adapting sampling method, which combines a Quasi-Monte Carlo sampling and gradient image, resulting in an invasive tumor detection probability map. The bottom table show the resilience to inter-institutional variability in terms of AUC performance from two trained models with HUP and UHCMC validated into CINJ data cohort.

Conclusions: We presented a feature learning framework for invasive tumor region detection in BCa WSI. This framework shows inter-institutional confident and reproducible results from three data cohorts. Additional experiments involving subtypes of invasive tumors are required.

149 Clinical Impact of ER/PR Levels in Patients With Luminal A and B/HER2-Negative Breast Carcinoma

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Background: Patients with breast carcinoma(BC) with positive estrogen and/or progesterone receptors(ER/PR) have better prognosis than those with negative tumors. Novel multigene assays allow the stratification of patients at different risk,but they are expensive and of limited availability.According to ASCO/CAP,tumors with≥1% positive nuclear staining by immunohistochemistry(IHC) for ER should be considered positive.Different expression levels may reflect the observed differences in survival. Therefore,the aim of our study in a large series of patients with BC diagnosed and treated at the same institution was to determine an ER/PR cut-off to select potential subgroups with different prognosis.

Design: We selected 893 patients with ER/PR-positive BC.Median clinical follow-up was 92 months (range 6-414).All slides were reviewed and formalin fixed paraffin-embedded block tissues were selected containing the highest amount of tumor.IHC was performed for ER,PR and HER2. The results of ER and PR were stratified in three groups according to nuclear percentages of positivity: ≥5% vs≥10% vs≥20%.HER2 status was confirmed by FISH/CISH in all 2+ and <10% 3+ cases.The results were statistically correlated with clinical-pathological factors and outcome.A p-value<0.05 was considered significant.

Results: We found ER<5% positive in 1%(9/893) of the tumors,<10% in 3.36%(30/893) and<20% in 6%(54/893) and for PR 3.9%(35/893),19% 169/893) and 23.4%(209/893) respectively.A higher significant correlation was detected with older ages,smaller tumor size,histological grade1,no necrosis and LVI,when ER and PR positivity cut-offs were established at 20% compared with 10% vs 5% (all p<0.05; Chi-square and Fisher tests).Survival analysis showed that the 5% cut-off defined better a group of patients with higher rate of recurrence:for ER <5%,only 60% did not recur vs 81% in ≥5% (p=0.006);and for PR, 60% vs 83% patients were free of disease (p<0.000). Similarly,only 60% of the patients were alive at last follow-up if ER <5% vs 86% in cases with ≥5% (p=0.005).For PR results, 63% of the patients were alive vs 88% with higher tumor levels (p<0.000) (Kaplan-Meier;log-rank test).

Conclusions: Our findings in a large series of patients with Luminal A and B/HER2-negative BC show that tumors with ER/PR-positive in≥20% nuclei are related with good clinical-pathological factors.Nevertheless,the 5%cut-off level defines better a distinct subset of ER/PR patients with higher risk of recurrences and/or mortality.Therefore, those patients would benefit of additional chemotherapy.

150 CD34-Negative Mammary Myofibroblastoma – A Study of Eight cases

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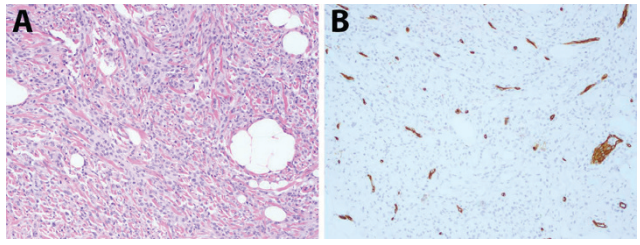
Background: The various morphologic variants of mammary myofibroblastoma (MM) hinder rapid identification of these tumors. However, their consistent immunoreactivity for CD34, regardless of their morphologic appearance, greatly aids in confirming the diagnosis. More recently, CD34 positive tumors including MM have been found to harbor monoallelic deletion of 13q14. The purpose of this study is to better characterize a rare subset of MMs that lacks CD34 expression.

Design: Eight cases of MM with reported negative CD34 immunoreactivity were identified in our surgical pathology and breast consultation files. For each case, the morphologic features and corresponding CD34, estrogen receptor (ER), and desmin immunostains were reviewed (TMD,SJS). In addition, a dual-color interphase FISH assay was performed on unstained paraffin slides to assess loss of the 13q14 region using the LSI RB1 probe and a control probe on chromosome 13 for each case.

Results: Seven tumors occurred in women; 1 occurred in a man (ages 41-85 years, median 49). Tumor size ranged from 0.4 to 2.5 cm (mean, 1.4 cm). Five tumors showed classic MM features - bland uniform spindle cells growing in fascicles with intervening bands of collagen and variable amounts of fat (**Figure 1A**). Of these, 1 case showed no staining for CD34 and 3 showed weak focal (<5% cells) staining (**Figure 1B**). The remaining case was dimorphic where ¾ of the tumor was of the myxoid variant and ¼ showed classic features. The myxoid areas were negative for CD34, whereas the classic areas were strongly positive. Of these 5 tumors, 1 showed deletion of 13q14 by FISH. 3 additional cases showed smooth muscle differentiation by morphology and immunohistochemistry and as such, were classified as the leiomyomatous variant. One of these did not show deletion by FISH; FISH is pending for the remaining 2 cases. 8/8 (100%) tumors showed positive staining with ER, 7 of which were moderate-strong and diffuse. Additionally, 7/8 (88%) showed diffuse staining with desmin.



Conclusions: MMs that lack or show weak, focal CD34 immunoreactivity can either show classic morphologic features or represent variants, particularly the leiomyomatous or myxoid types. These rare examples can further harbor deletion of 13q14 despite lacking CD34 expression.

151 Cystic Neutrophilic Granulomatous Lobular Mastitis: Further Clinical and Pathological Characterization of an Under-Recognized Entity Based on Eleven Cases

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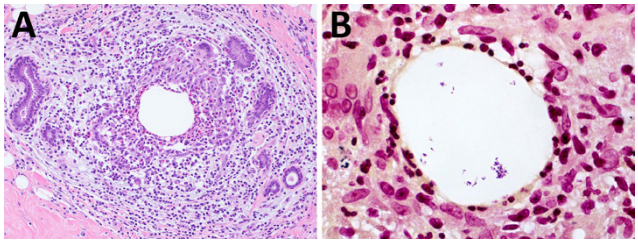
Background: Cystic Neutrophilic Granulomatous Lobular Mastitis (CNGLM) is a recently described Corynebacterium-associated disease process (Am J Clin Pathol 2011;136:424) which remains relatively poorly characterized.

Design: An electronic search of our departmental database over a 7-year period (2006-2013) for breast specimens with "granulomatous mastitis" and "abscess" was conducted. All such cases were subjected to histopathological review. Only cases that exhibited characteristic features of CNGLM were included. Acid-fast bacilli (AFB), fungal (GMS), and Gram stains were performed on all cases. All pertinent imaging studies were reviewed by a breast radiologist.

Results: Eleven cases of CNGLM were identified. All patients were women who had presented with a palpable breast mass (age, range: 25-49 years, median: 34). Two patents had bilateral metachronous disease. Seven were parous, and 6 had breastfed. Imaging studies in 11 patients showed irregular masses (1.7-5.7 cm) with a BI-RADS score of 5 (highly suggestive of malignancy) in 2 patients. CNGLM showed lobulocentric suppurative granulomas with epithelioid histiocytes, Langhans giant cells, and mixed inflammatory cell infiltrate (Figure 1A). Clear vacuoles surrounded by neutrophils contained Gram-positive bacilli in 5/11 cases (Figure 1B). AFB and GMS stains, and all microbiological culture studies, were negative in all cases. Signs and symptoms worsened (with erythema, draining sinus) following initial needle core biopsy in 4 patients. Six patients were treated with antibiotics, 5 patients were treated with both antibiotics and surgery, and 1 was treated surgically. The disease process resolved in 1 to 6 months.



Conclusions: CNGLM presents as a unilateral irregular mass with characteristic histopathology. The mass may radiologically mimic malignancy. Needle core biopsy can aggravate symptoms. Gram-positive bacilli can be demonstrated in some cases. Microbiology cultures are often negative which may be due to the fastidious nature of these organisms. The disease resolves in a few months with conservative management.

152 Positive GATA-3 and FOXA1 Expression Is Useful To Differentiate Breast Carcinoma From Other Carcinomas

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Background: It is important to differentiate breast cancers from other carcinomas in daily practice, as morphology may overlap, especially in the metastatic setting. GATA-3 has been shown to be a useful marker to identify breast carcinoma. However, few studies have examined the extent of GATA-3 reactivity in other carcinomas. Likewise, the expression of FOXA1, an essential protein in the estrogen receptor pathway, has not been well studied in breast and other carcinomas. We examined GATA-3 and FOXA1 expression in breast and 11 other different types of carcinomas.

Design: Tissue microarrays composed of ER+/Her2-, Her2+ and triple negative breast carcinomas, melanoma and other adenocarcinomas including hepatocellular, colonic, pancreatic, gastric, endometrial, lung, prostatic, renal cell, urothelial, and ovarian serous carcinoma were analyzed. Immunohistochemical studies were performed using GATA-3 primary antibody (1:50 dilution, Biocare, CA) and FOXA1 primary antibody (1:50 dilution, Millipore, CA). The primary antibodies were detected with EnVision+ dual link kit (Dako). Any nuclear staining of GATA-3 or FOXA1 was considered positive.

Results: 1) Among the carcinomas stained with GATA-3: 115 of the 119 (96.6%) ER+/Her2-, 66 of 80 (82.2%) Her2+, and 13 of 51 (25.5%) triple negative breast carcinomas were positive. Of other carcinomas stained for GATA-3, 56 of 79 (70.9%) urothelial carcinomas were positive and none of the remaining carcinomas were immunoreactive. 2) Among the carcinomas that stained for FOXA1: 127 of 132 (96.2%) ER+/Her2- breast carcinomas, 80 of 97 (82.5%) Her2+ breast carcinomas, 7 of 57 (12.3%) triple negative breast carcinomas, 42 of 48 (87.5%) prostatic adenocarcinomas and 4 of 75 (5.1%) urothelial carcinomas were positive. None of the other carcinomas were immunoreactive.

Conclusions: Our data show a relatively high specificity of GATA-3 expression in ER+/Her2 negative and Her2+ breast carcinoma and urothelial carcinoma. High specificity of FOXA1 staining in ER+/Her2- breast carcinoma, Her2+ breast carcinoma and prostatic adenocarcinoma was observed. Combination of GATA-3 and FOXA1 staining is useful to differentiate breast carcinoma from other carcinomas including urothelial carcinoma. The relatively low expression rate of GATA-3 in triple negative breast carcinoma limits its application in the diagnosis of triple negative breast cancers. FOXA1 may be a useful marker to differentiate urothelial from prostatic adenocarcinoma; it is of little utility in the diagnosis of triple negative breast carcinomas.

153 Influence of "In Vitro" and "In Vivo" Contexts on the Expression of Cancer Stem Cell Markers in Breast Cancer-Derived Cell Lines

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Background: Recently, in a new paradigm of carcinogenesis, cancer stem cells (CSCs) have been regarded as responsible for the origin, morphologic heterogeneity, self-renewal and therapeutic resistance of malignant neoplasms. As such, they have become an important target in pathophysiological/pharmacological studies of cancer. Currently, one of the main obstacles to the study of CSCs is the lack of reliable biomarkers. Although there seems to be some consensus regarding what should be considered typical CSC markers in *in vitro* studies, there is very poor agreement when these are contrasted to *in vivo* studies based on human tumor tissue.

Design: Using three distinct breast cancer-derived cell lines, we investigated the expression of CSC markers in two biological contexts reflecting differences in microenvironment (*in vitro vs. in vivo*), and in two immunophenotyping techniques (flow cytometry *vs.* immunocytochemistry).The expression of CD24, CD44, CD133, EpCAM and ALDH1/ALDEFLUOR was assessed in cell and tissue samples (orthotopic xenotransplants from NOD/SCID mice) of MCF-7, MACL-1 and MGSO-3 cell lines, using flow cytometry and immunocytochemistry (n= 5 samples/group).

Results: Data provided by flow cytometry was not found to be reliable, since most CSC markers were negative in this technique, regardless of cell line or context. Data provided by immunocytochemistry showed marked variability in the prevalence of positive samples for EpCAM, CD24, CD44, CD133 and ALDH-1 across cell lines and biological context. In *in vitro* samples (cell-blocks), EpCAM presented the greatest frequency of positive samples (50-80% across cell lines) and of positive cells per sample (mean frequency of positivy: 75%). By contrasting cell-blocks with xenografts, however, we found a significant decrease in the frequency of positive cases ($p<0.01$; Fisher's Exact Test) and of positive cells per sample ($p<0.001$; Paired T test) in tissue samples, for most CSC markers. The only exception was EpCAM in MCF-7 cell line, which was increased in xenografts.

Conclusions: Our data confirm the role of microenvironment in the maintenance of CSCs, further indicating that it may be an important source of variability in the expression of CSC markers across independent studies. In addition, our results suggest that immunocytochemistry may be more reliable than flow cytometry in the detection of cancer stem cells by immunophenotyping, in both cell and tissue samples.

154 Cancer Stem Cells (CSCs) Are Increased in Pregnancy Associated Breast Carcinomas (PABC)

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Background: Cancer stem cells (CSCs) have been shown to play an important role in initiation and progression of human malignancies including breast carcinomas. In breast cancer they are thought to be associated with higher grade tumors, triple negative (TN) or HER2 phenotype and poor prognosis. Pregnancy associated breast carcinoma (PABC) arises during or after pregnancy, is often TN and is also associated with poor prognosis. However, to date, CSCs in PABC have not been adequately evaluated. In this study we examined the expression of ALDH1, CD44 and CD24 CSC markers and correlated their expression with molecular and histologic characteristics of PABC.

Design: Our patient population consisted of 23 patients diagnosed with pregnancy associated breast cancer during or within 2 years of pregnancy (mean age: 35.8, range: 26-48). Age-matched nulliparous women with a diagnosis of breast cancer served as controls (mean age: 37.5, range: 29-51). Tumors were immunohistochemically stained for ALDH1, CD44 and CD24. Pathologic characteristics included histologic type, tumor grade, tumor size, presence of lymphatic vascular invasion, lymph node status, and breast tumor markers (ER, PR, HER2, p53).

Results: Six (26.1%) PABC cases but only 1 (6.7%) control case showed ALDH1 expression. Of interest, all the ALDH1+ tumors were high grade. In the ALDH1+ PABC group, 3 (50%) tumors were TN, 2 (33.3%) HER2 and only 1 (16.7%) of luminal subtype. In contrast, the majority of the ALDH1 negative cases were of luminal subtype; 10 (58.8%) in the PABC group and 14 (100%) in the control group. Five (83.3%) of the ALDH1+ PABC cases had lymph node metastasis and 3 (50%) had lymphovascular invasion (LVI) compared to 8 (47%) cases of lymph node metastasis and 5 (29.4%) in ALDH1- population in PABC. The $CD44^{+}/CD24^{-/Low}$ phenotype was seen in 8 (34.7%) cases of PABC and 6 (40%) cases in the control group; however, no correlation was seen with any clinical or histologic parameters.

Conclusions: 1. More than a quarter of PABCs express ALDH1. 2. ALDH1+ PABC tumors tend to be high grade, with TN or HER2 phenotypes and are more likely to have lymph node metastasis and LVI. The results of our study suggest that CSCs are present in PABC in increased numbers and their presence may contribute to the poor prognosis in PABC. Additional studies are underway to expand these findings and further characterize the role of CSCs in these challenging breast tumors.

155 Pathological Features of Atypical Ductal Hyperplasia Diagnosed on Core Needle Biopsy Predictive for Upgrade on Excision

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Background: Atypical ductal hyperplasia (ADH) is defined as the proliferation of monomorphic, evenly placed epithelial cells involving terminal ducto-lobular units (TDLU) that does not meet the criteria for low-grade DCIS, due to partial involvement of TDLU, or complete involvement of only a limited number of TDLU. Current treatment of ADH diagnosed on core needle biopsy (CNB) consists of surgical excision (SE) to exclude DCIS or invasive carcinoma. Our goal is to identify pathological features of ADH on CNB that predict upgrade to carcinoma on SE.

Design: We performed a retrospective review of cases diagnosed as ADH on CNB between January 2009 and January 2011. A total of 50 cases from 47 patients were identified and analyzed for histological features that may be predictive of carcinoma upgrade. Variables analyzed included nuclear features, presence of mitoses and calcifications, architectural subtype, size of the largest focus of ADH, number (#) of cores positive for ADH, # of foci of ADH, and percentage (%) of cores positive for ADH. Two-tailed Student's t-test was performed to identify statistically significant differences between means and the Chi Square test was used for categorical variables.

Results: A total of 18 cases (36%) were upgraded to carcinoma on subsequent surgical excision. 10 (56%) of these cases were upgraded to DCIS and 8 (44%) were upgraded to invasive carcinoma. Our findings are summarized in the following table:

Histological feature	Upgrade	No-upgrade	p value
Nuclear features*	9 (50%)	8 (25%)	0.06
Mitoses present	0 (0%)	2 (7%)	0.28

Calcifications present	13 (72%)	24 (75%)	0.83
Presence of cribriform architecture	16 (89%)	28(88%)	0.88
Presence of micropapillary architecture	1 (5%)	6 (19%)	0.20
Average (Avg) size of the largest focus (mm)	2.61	1.27	0.007
Avg # cores involved by ADH	2.055	2.62	0.18
Avg # of foci involved by ADH	2.16	2.68	0.28
Avg % cores involved by ADH	37	30	0.29

*Nuclei with mild-moderate variability in size, shape and placement, variably coarse chromatin, and variably prominent nucleoli.

Conclusions: 36% of cases of ADH diagnosed on CNB were upgraded to carcinoma on subsequent SE. The histological feature that was found to predict carcinoma upgrade on excision is the size of the largest focus of ADH on CNB. Nuclear features have a trend towards upgrade to carcinoma on subsequent SE.

156 Molecular Subtypes of Breast Cancer in Chromosome 17 Polysomy

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Background: Breast cancers can be divided into molecular subtypes (Luminal A, Luminal B, HER2, and Triple Negative) which have prognostic and therapeutic implications. The human epidermal growth factor receptor 2 (HER2) oncoprotein, a known adverse prognostic factor is overexpressed in about 20% of breast cancers and HER2 gene amplification is responsible for protein overexpression in about 90% of these cases. Studies have found that unamplified increased chromosome 17 copy number, commonly referred to as polysomy, is also associated with several adverse prognostic factors. The aim of this study is to examine the distribution of molecular subtypes of breast cancer in cases with increased chromosome 17 copy number.

Design: Cases of invasive breast carcinomas from 1997 to 2013 that had chromosome 17 polysomy (≥ 3.0 CEP17 copies/cell) by fluorescence in situ hybridization (FISH) for HER2 were included. These cases were then classified into molecular subtypes as follows: Luminal A [ER+ and/or PR+, HER2-, and Ki67 <14%]; Luminal B [ER+ and/or PR+ and HER2+ or ER+ and/or PR+, HER2-, and Ki67 >14%]; HER2 [ER-, PR-, and HER2+]; and Triple Negative [ER-, PR-, and HER2-]. HER2 status by FISH was determined by both the 2007 and 2013 ASCO–CAP guidelines. HER2 results based on the 2013 guidelines (excluding equivocal cases) were used for determining the molecular subtypes.

Results: Of the 1846 cases of invasive breast carcinoma with HER2 FISH results, 389 cases (21%) had increased chromosome 17 copy numbers. Using the 2007 vs. 2013 HER2 guidelines, 202 cases (51.9%) had concordant FISH results, while 187 cases (48.1%) were discordant. Of the discordant cases, HER2 status changes were as follows: negative to equivocal (153 cases, 81.8%), negative to positive (23 cases, 12.3%) and equivocal to positive (11 cases, 5.9%). Thus, 34 additional cases (18.2%) were HER2 positive by the 2013 guidelines.

Molecular subtypes of the polysomy cases were as follows: Luminal type A or B (192 cases, 81.4%), Triple negative (26 cases, 11.0%), and HER2 (18 cases, 7.6%). Within the luminal category, 102 cases were able to be further classified based on Ki-67 results: Luminal B (80 cases, 78.4%) and Luminal A (22 cases, 21.6%). Of the 80 Luminal B cases, 18 cases were ER+ and/or PR+, HER2-, and Ki67 >14%.

Conclusions: The majority of cases with increased chromosome 17 copy numbers are of molecular subtypes with more adverse prognosis: Luminal B (78.4%), Triple negative (11.0%) and HER2 amplified (7.6%). In addition, 34 cases (18.2%) would be eligible for HER2 therapy according to the 2013, but not the 2007 HER2 guidelines.

157 Use and Outcome of Receptor Analysis for Multiple Foci in Synchronous Ipsilateral Invasive Breast Carcinoma

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Background: For patients with multiple synchronous invasive breast carcinomas, CAP/ASCO guidelines recommend estrogen receptor (ER), progesterone receptor (PR) and HER-2/*neu* analysis on "at least one of the tumors, preferably the largest." In practice request for testing additional foci is variable.

Design: Cases of multifocal ipsilateral invasive breast carcinoma, defined as discrete foci with intervening benign tissue (N=157; 6/04-6/14), were assessed for number of foci, clinicopathologic features and treatment. ER/PR were defined as negative (<1%), weak (1-10%) and positive (>10%). HER-2/*neu* was defined as negative, equivocal and positive by staining &/or FISH per CAP/ASCO guidelines.

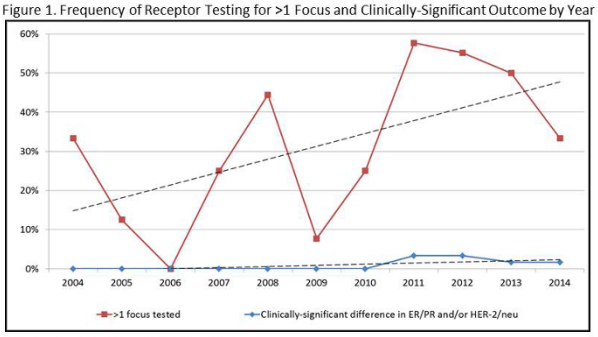
Results: 102 (65%) had 2 and 55 (35%) had >2 foci of invasive carcinoma (range 2-9). 92 (58.6%) were T1 and 65 (41.4%) were T2/T3. 75 (47.8%) were N0, 48 (30.6%) N1 and 32 (20.3%) N2/N3. Lumpectomy 50 (31.8%) and mastectomy 107 (68.2%) were final surgeries. 84 (53.5%) had SLN only and 71 (45.2%) ALND. Post-surgical therapy included: 84 (54.2%) radiation, 121 (78.1%) endocrine, 67 (43.2%) chemotherapy & 19 (12.3%) Herceptin. Largest tumor foci had the following histology: 85 (54.1%) IDC, 27 (17.2%) IDC-L, 40 (25.5%) ILC & 5 (3.2%) other; and grade: 27 (17.2%) 1, 89 (56.7%) 2 & 41 (26.1%) 3.

Categorical differences for cases with >1 focus tested and frequency of testing >1 focus and clinical significance (treatment change) by year are shown below. Cases with clinical significance (6/60, 10%) had low ER/PR% (1), equivocal HER-2/*neu* (1), different histology (2) and grade (3).

Table 1. Differences in Receptor Profiles in Patients with >1 Focus Tested

	Neg ↔ Weak/Equiv	Weak/Equiv ↔ Pos	Neg ↔ Pos
ER (N=60)	0	1 (1.7%)	2 (3.3%)
PR (N=60)	5 (8.3%)	6 (10%)	1 (1.7%)
HER-2/*neu* (N=59)	1 (1.7%)	0	4 (6.8%)

Figure 1. Frequency of Receptor Testing for >1 Focus and Clinically-Significant Outcome by Year



Note: 2004 (7 mos.) and 2014 (5 mos.) are partial

Conclusions: Receptor testing for additional foci in multifocal breast carcinoma is increasing, with minimal change in patient management. Clinically-significant differences in receptor profiles were only identified in cases with low ER/PR expression, equivocal HER-2/*neu* and different histology &/or grade; in these cases testing additional foci appears warranted.

158 Massively Parallel Sequencing Analysis of Infiltrating Epitheliosis of the Breast

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Background: Infiltrating epitheliosis (IE) is a rare complex sclerosing lesion, characterized by infiltrating ducts and duct-like structures immersed in a scleroelastotic stroma and filled with cells having architectural and cytological patterns reminiscent of those of usual ductal hyperplasia (UDH). The histologic features of IE and the lack of myoepithelial cells, particularly at the periphery of the lesions, raise the concern that IE may constitute a form of low-grade infiltrating neoplasm. In this study we sought to define the molecular characteristics of this unique lesion.

Design: Cases of IE were retrieved from the consultation files of one of the authors (IOE). Only samples independently classified by three pathologists as IE were included. Nine IEs, adjacent breast lesions (1 UDH, 1 papilloma, 1 micropapillary DCIS, and 2 low-grade adenosquamous carcinomas), and corresponding normal breast tissue from each case were microdissected, and subjected to massively parallel sequencing analysis targeting all coding regions of 254 genes recurrently mutated in breast cancer or involved in DNA repair-related pathways.

Results: Recurrent mutations in PI3K pathway genes were found in all 9 cases of IE. Specifically, 8 of 9 cases harbored *PIK3CA* hotspot mutations (the H1047R mutation in 6 cases, E545K in 1 case, and E542K in 1 case). The *PIK3CA* wild-type case displayed a *PIK3R1* (KL379K) codon deletion. In a case with IE, micropapillary DCIS, and low-grade adenosquamous carcinoma the same *PIK3CA* (H1047R) and *SF3B1* (K700E) hotspot mutations were found in all components. In a case of IE, a papillary lesion and a low-grade adenosquamous carcinoma, the first two components shared the same *PIK3CA* mutation (H1047R); whereas the low-grade adenosquamous carcinoma did not harbor any somatic mutation in the genes analyzed.

Conclusions: Recurrent somatic mutations affecting PI3K pathway genes were identified in all IEs, providing direct evidence that these lesions are clonal and neoplastic rather than hyperplastic. In one case, the IE may have constituted the substrate from which the DCIS and adenosquamous carcinoma originated. The landscape of somatic genetic alterations found in IEs is similar to that of radial scars/complex sclerosing lesions, suggesting that IEs may represent one end of the spectrum of these lesions.

159 Metaplastic Carcinoma of the Breast Is More Aggressive Than Triple-Negative Breast Cancers

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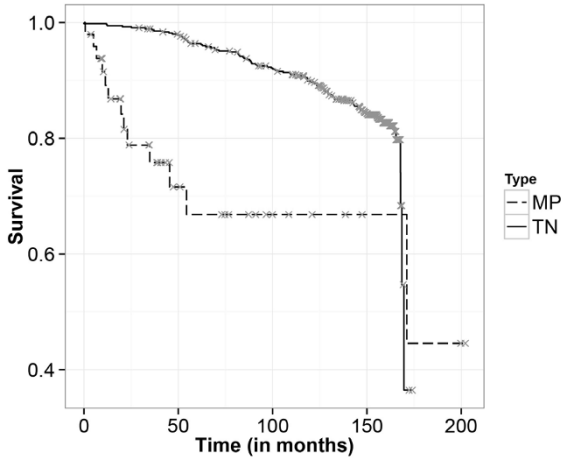
Background: Metaplastic carcinoma (MC) is a heterogeneous group of cancers that encompasses multiple subtypes. The aim of this study is to review the morphologic features and clinical characteristics of this tumor and subclassify MC based on clinical outcome, then to compare its clinical outcome with triple negative (TN) breast cancers from a single institution.

Design: We reviewed 85 reported MC cases from our institution between 1992 and 2013 yielding 52 cases, three patients lost to follow-up. The percentage of the components that had distinctive morphology, squamous cell carcinoma (SCC), matrix producing (MP), spindle cell high grade (SHG), spindle cell low grade (SLG), and low grade adenosquamous (LGAS) were recorded. Tumor was designated as mixed when at least two types were identified and each comprised at least 10% of the tumor. Tumor was graded using Nottingham grading system. Tumor size, lymph node status, stage and biomarkers profile was extracted from the pathology reports. Standard clinical information including patient's age, race, menopausal status, type of therapy

(hormonal, chemo- and radiation) and survival data was extracted from the clinical reports. The clinical and pathological data for 558 TN cases was extracted from the breast cancer database.

Results: There were 20 (38.6%) MP, 15 (28.8%) SCC, 10 (19.2%) SHG, 2 (3.8%) SLG, 2 (3.8%) LGAS, and 3 (5.8%) mixed. ER was positive in 6 (12.5%), PR in 7 (14.6%) and HER2 in 3 (6.5%) cases. The number of patients who died from disease was 13 (26.5%) with MC and 96 with TN (20.9%) (p=<0.001) (figure1). The median follow up was 38.6 and 157.8 months respectively. Only postmenopausal status was predictive of death due to disease (p=0.036). There was no significant survival difference between MP, SCC or SHG. However, two patients with LGAS and 2 with SLG had no tumor recurrence or death due to disease after follow up time from 3-years to 16-years.

Conclusions: We present a relatively large number of cases with a long clinical follow-up. We found that metaplastic carcinoma is more aggressive than TN cancers. Two WHO-recognized MC subtypes, SLG and LGAS, should be recognized as separate entities as they have distinctively better clinical outcome. More cases, particularly from these two types are needed to affirm our interpretation.



160 RUNX2 Expression as a Potential Prognostic Marker in Invasive Ductal Breast Carcinoma

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Background: The Runx family of transcription factors are implicated in cancer progression, both positively and negatively. Recent studies assigned a role for Runx2 in promoting breast cancer metastasis. However, the role of Runx2 in the early stage of breast carcinoma, and its association with clinical outcome is still unknown.

Design: We aimed at assessing the clinicopathological significance of Runx2 expression in a cohort of 84 invasive ductal breast carcinomas. The correlation between the nuclear Runx2 labelling index (LI) in breast carcinoma cells and clinicopathological parameters was assessed. To study the association of Runx2 with patients' outcome, beside treating it as a continuous variable, Runx2 was categorized by the its median value. Optimal cut-off points were determined, by ROC curve analysis, for prediction of disease free survival (DFS) and overall survival (OS). Multivariate Cox regression models were built and best subset regression models were identified to predict the probability of recurrence, metastasis and/or death. Validation was done; and based on the "Predicted R^2", the best three models were identified. The interaction between Runx2 and other clinicopathological terms was also tested.

Results: Runx2 LI associated significantly only with positive Her-2 status, and did not correlate significantly with other clinicopathological parameters. Although, Runx2 LI, continuous form and when categorized by its median value (65), did not correlate significantly with DFS and OS, yet, after it was categorized using optimal cut-off points, patients with Runx2 LI >45% showed significantly higher event rate and shorter DFS, (P=.047); while patients with Runx2 LI >40% showed significantly shorter OS, (P=.050). Moreover, Runx2 LI effectively contributed to the models built to predict DFS and to a lesser extent to the models built to predict OS. Regarding DFS, the diagnostic performance of Runx2 did not differ according to the clinicopathological characteristics of the tumor. However, among stage IV tumors, the interaction term between Runx2 LI and ER status was a significant predictor of overall survival. In this model, Runx2 was a significant predictor.

Conclusions: Runx2 has a role in the biological behavior and affects outcome of invasive ductal breast carcinomas, thus, its inhibition might open a new strategy for therapy. The predictability of Runx2 for OS in stage IV tumors differs with difference of ER status. The pattern of this difference was not studied as the sample size was not sufficient to allow pattern testing.

161 Increase of Equivocal In Situ Hybridization Results in 2013 ASCO/CAP Guidelines for HER2 Testing in Invasive Breast Cancer: Comparison With the 2007 Criteria

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Background: The ASCO and CAP have updated guideline recommendations for human epidermal growth factor receptor 2 (*HER2*) testing in breast cancer. Concerning *in situ* hybridization (ISH), positivity is now defined as *HER2* copy number ≥6.0 signals/cell, or *HER2*/CEP17 ratio ³2. The equivocal category is defined using a copy number of >4 to <6 signals/cell per cell and a ratio of <2. Our aim was to compare ISH criteria of ASCO/CAP update (2013) with the previous guidelines (2007) in routine practice.

Design: A total of 622 FFPE breast tumor samples (Jan 2013-Sept 2014) were included. *HER2* amplification was assessed by SISH (Ventana) or FISH (AbbotMolecular) as the initial test. We compared *HER2* ISH results according to 2007 and 2013 ASCO/CAP recommendations. In 96 cases with a discordant and/or equivocal 2013 criteria ISH result, IHC was performed as alternative test using *PATHWAY anti-HER2 antibody* (Ventana).

Results: In 88/622 samples (14%) a discordant ISH result was found. In most of them (58/88; 66%) it changed from negative to equivocal. 26/88 samples (29%) became a new positive result; 18 were equivocal cases by 2007 criteria, with ≥6.0 signals/cell and ratios between 2 and 2.2. Only 4 equivocal ISH cases became negative according new scoring (4/88; 5%). Regarding 2013 criteria, 66 assessments were equivocal (86% of which were polysomic). IHC data were available for 60/66 samples: 67% were negative (0 or 1+) and 30% 2+, whereas only two cases were positive (3%). None of the 19 new positive ISH assessments with available IHC showed positivity by the latter technique (6 were 2+), but negative IHC was confirmed in 3/3 new negative ISH results.

Conclusions: 1. Using the HER2 update ISH recommendations, we observed an increased number of inconclusive cases (5% vs 11%), which need ISH retesting on another sample or performing an alternative test (IHC). Most of these cases showed polysomy (4-5 copies) of chromosome 17 and were negative by IHC; 2. A positive cases increase was observed (18% vs 23%), mainly due to those with ≥6.0 signals and to the decrease in the ratio from ³2.2 to ³2. However, no correlation between new ISH criteria and IHC was observed; 3.Among the 2013 criteria equivocal ISH results, 3% were confirmed as positive by IHC (3+), which would be considered as a false-negative by the 2007 criteria.

162 Low Incidence of Invasive Recurrence After Long-Term Follow-Up of Patients With Low-Risk DCIS Treated With Breast Conservation: A Single Institution Follow-Up Study

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Background: For more than 20 years an overwhelming body of evidence has demonstrated ductal carcinoma in situ (DCIS) to be a surgical disease appropriately managed by breast conservation (BCT) provided that negative margins can be confidently achieved while the role of radiation therapy (XRT) has become increasingly controversial.

Design: Clinicopathologic features of patients with DCIS who participated in a prospective breast cancer repository database at Vanderbilt Ingram Cancer Center, Nashville, TN were correlated with outcome.

Results: 44 patients diagnosed with DCIS between 5/1998 and 2/2004 were identified. DCIS was graded as high (HG, n = 13), intermediate (IG, n = 20) and low nuclear grade (LG, n = 11). 37 women had more than 5-year follow-up (average 10.3 years; range 5.3 to 13.6 years), and 24 of them were treated by breast conservation, (BCT) while 10 women underwent total mastectomy (TM). Among women treated by TM, one woman with extensive DCIS experienced a locoregional invasive recurrence (8/23 axillary lymph nodes) 3 years later and another developed DCIS in the contralateral breast 3 years later. Both had HG DCIS and had not received XRT or tamoxifen (TAM). 14 of 23 patients who underwent BCT also received XRT, including all HG cases (5/5), and none experienced recurrence. The only ipsilateral invasive recurrence occurred in a woman 8 years after a diagnosis of IG DCIS. She also had DCIS in the contralateral breast and did not receive XRT or TAM. Another woman had an ipsilateral recurrence of IG DCIS 6 years after her original diagnosis. She was successfully treated by TM and was alive with no evidence of disease at last follow-up 12.3 years after her original DCIS diagnosis. Among 9 women with < 5yrs follow up, 3 were treated by TM and 6 by BCT (2 also had XRT). To date only 1 women who underwent TM experienced invasive recurrence (1 year after her extensive LG DCIS diagnosis). To date there are no recurrences among women undergoing BCT with or without XRT in the group with less than 5 year follow-up.

Conclusions: Invasive recurrence rates following a diagnosis of DCIS at our institution are very low. The decision to use XRT is influenced by grade and size. Two of 3 invasive recurrences occurred in women who had undergone TM, raising the possibility of residual unsampled disease. Most women with smaller LG and IG DCIS can be successfully treated by BCT alone provided negative margins have been confidently achieved.

163 Targeted Next Generation Sequencing Identifies Variants of Standard Biomarkers and Potentially Actionable Alterations in a Majority of Advanced Breast Cancer Patients

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Background: Molecular tumor profiling is becoming increasingly important in the management of oncologic patients. Targeted next generation sequencing (T-NGS) comprehensive cancer gene tests utilizing formalin fixed paraffin embedded (FFPE) clinical samples allows molecular characterization of genes with known or potential therapeutic and prognostic importance in cancer.

Design: We correlated clinicopathologic information on patients with metastatic breast cancer (BC) or refractory locoregional recurrence with potentially actionable genetic alterations detected by a commercial T-NGS assay sequencing the coding regions of 236 genes and 47 introns (19 additional genes). The detected alterations were classified as: actionable with FDA approved therapies in BC, actionable with FDA approved therapies in other cancers, an eligibility criterion for a clinical trial/potentially efficacious as an investigational agent or a variant of unknown significance.

Results: Between 11/2013 and 8/2014 84 FFPE samples were tested by T-NGS, identifying 420 known somatic alterations in 95 genes, including at least one potentially actionable alteration in 99% of samples (median 5 alts/tumor[range 0-13]) including alterations predicting sensitivity to approved BC therapies in 61% of patients. 25% of patients received genotype-directed treatments based on the test results, mostly in clinical trials. Although the average number of alterations/pt and alteration type (MUT,AMP, DEL) did not differ significantly among breast cancer subtypes, the proportion of genes within specific dysregulated pathways and individual genes varied. The most frequently altered pathways were p53 (70%:57% ER+, 70% HER2, 92% TNBC), RTK/GF (57%:42% ER+, 95% HER2+, 50% TNBC), cell cycle (55%:55% ER+, 50% HER2+, 58% TNBC), and PIK3/mTOR (53%:52% ER+, HER2+50%, 58% TNBC). Among ER+ (n = 40), HER2+ (n = 20) , and TNBC (n = 24) tumors, the most frequent alterations were TP53 mutations (42%, 70%, and 87%), PIK3CA mutations (35%,30%,and 29%) and ERBB2 amplification (0%, 70%, and 8%). Variants of standard biomarkers including 3 HER2 mutations (1 HER2+ and 2 TNBC) and 4 ESR1 mutations were also identified which impacted treatment decisions.

Conclusions: Mutational profiling using T-NGS identified potentially actionable alterations in a majority of advanced BC patients, providing novel yet rational therapeutic options and facilitating clinical trial enrollment. In the future NGS results will be used to guide therapy in increasing numbers of BC patients.

164 Prevalence of Androgen Receptor Expression in Triple Negative Breast Cancers According To BRCA1 Mutation Status

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Background: Triple negative breast cancers (TNBC), defined by lack of estrogen receptor, progesterone receptor and HER2 expression, comprise about 15% of all breast cancers but are much more common in women with germline *BRCA1* mutations. Recent studies have demonstrated considerable heterogeneity of TNBC at the gene expression level; six molecular TNBC subtypes have recently been described. In particular, while some TNBC have basal gene expression signatures others resemble luminal tumors and demonstrate high levels of expression of androgen receptor (AR) mRNA and nuclear AR expression by immunohistochemistry. The frequency of AR positivity in TNBC in relation *BRCA1* mutation status has not been well studied. However, given the availability of clinical trials evaluating the efficacy of AR targeted therapies in TNBC and efforts to define appropriate systemic therapy for *BRCA1*-associated TNBC, the relationship between AR expression and germline *BRCA1* mutation status in TNBC is of interest.

Design: We studied 197 TNBC: 79 (40.1%) from women with germline *BRCA1* mutations (*BRCA1*+ TNBC) and 118 (59.9%) from women without a *BRCA1* mutation (sporadic TNBC). Pathologic features evaluated included histologic type, grade, lymphovascular invasion (LVI), and lymphocytic infiltrates (LI). Tissue microarrays (TMA) were constructed from these tumors (three 0.6mm cores/case) and TMA sections were immunostained for AR as well as for CK5/6, CK14 and EGFR (to define TNBC with basal features).

Results: *BRCA1* carriers with TNBC were significantly younger (43.4 ±9.1 years) than those with sporadic TNBC (50.9 ±10.8 years; p<0.001). There were no significant differences in histologic type, grade, LVI or expression of CK14 or EGFR between *BRCA1*+ TNBC and sporadic TNBC (all p>0.19). LI was significantly more frequent in *BRCA1*+ than in sporadic TNBC (35.9% vs 23.4%; p =0.02). Among all TNBC, 17.8% were AR-positive (defined as at least 1% of cells staining for AR). Sporadic TNBC were significantly more often AR-positive than *BRCA1*+ TNBC (p=23.7% vs 8.9%; p=0.008). Conversely, *BRCA*1+ TNBC were significantly more often CK5/6-positive than sporadic TNBC (71.8% vs 54.2%; p=0.02).

Conclusions: Sporadic TNBC are significantly more often AR-positive than *BRCA1*+ TNBC whereas *BRCA*1+ TNBC are significantly more often CK5/6-positive than sporadic TNBC. These results confirm the heterogeneity of TNBC and, in particular, indicate that AR expression in TNBC varies significantly with *BRCA1* mutation status.

165 Reproducibility of Oncotype Dx Recurrence Score Using Magee Equations in Breast Cancers

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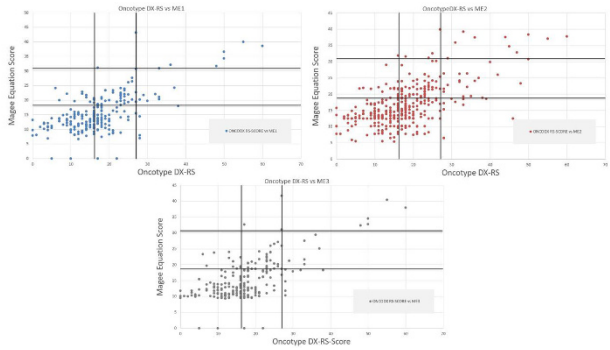
Background: Laboratory testing accounts for 10% of health care expenditure in the US. Many oncologists base chemotherapy decisions for patients with estrogen receptor (ER) positive lymph node negative breast cancers on the recurrence score (RS) from Oncotype DX Genomic Health Inc (GHI). The Magee equations (ME) use standard pathologic & immunohistochemical (IHC) [receptors, MIB1 proliferation index (PI)] parameters to estimate the RS. Motivated by the imperative of healthcare cost containment, we studied the concordance between GHI-RS with online ME RS in breast cancer patients from a large integrated health system to see if the ME method can be used as an alternative to the commercial test.

Design: We studied breast carcinomas from a single institution submitted for GHI-RS over a period 4 years. Pathologic parameters included tumor size, Nottingham grade, ER, PgR, Her2 & MIB1 PI. ER, PR were semi-quantitatively scored, an H-score with score >10 considered a positive. Her2 was scored using published ASCO/CAP guidelines. MIB-1 was scored as % positive tumor nuclei [low (<14%) & high (>14%)]. ME scores were generated using the online tool. Pearson correlations between GHI-RS vs ME RS were calculated.

Results: A total of 371 cases of breast carcinomas were identified. The average tumor size was 1.68 cm (SD: 0.97, range: 0.3-10 cm, median: 1.5 cm). There were 125/371 (33.7%) grade 1, 199/371 (53.6%) grade 2, 47/371 (12.7%) grade 3 tumors. There were 369/371 (99.5%) ER+, 2/371 (0.5%) ER- , 343/371 (92.5%) PgR+, 28/371 (7.5%) PgR-, 353/371 (95.2%) HER2-, 3/371 (0.8%) HER2-indeterminate, 15/371 (4.0%) HER2+ tumors. The GHI-RS included 200/371 (53.9%) low RS, 143/371 (38.5%) intermediate RS, 28/371 (7.5%) high RS. Pearson correlation between different ME & GHI-RS were: ME1=0.69; ME2: 0.62, ME3: 0.70.



Conclusions: Overall there was moderate to strong correlation between GHI-RS & all three MEs in our cohort. When reclassifying ME & GHI-RS into 3 groups (low, intermediate, high), the ME score did not precisely predict the GHI-RS. For high risk tumors, ME is just as sensitive as GHI-RS. This pilot study strongly warrants a longitudinal study of the actual survival / recurrence of our patients & its correlation with the pathologic parameters used to generate ME.

166 Can Histopathologic Features of Duct Cell Carcinoma In Situ of the Breast (DCIS) Be Used To Predict Oncotype DX DCIS Score

Susan Fineberg, Jana Fox, Nella Shapiro, Adriana Knopfelmacher Couchonal. Montefiore Medical Center, Bronx, NY.

Background: The Oncotype DCIS score is a multigene assay used to determine recurrence risk in pts with DCIS treated with excision without radiation (RT). It measures genes involved in proliferation, progesterone receptor and GSTM1. We looked at histopathologic features of cases of DCIS with an Oncotype Score to determine if these could be used to predict score.

Design: This study included 46 pts with DCIS and an Oncotype DCIS score. We used our clinical information system to determine clinicopathologic features. We reviewed H&E slide from the block which was sent for testing when available. We counted mitotic figures in all ducts with DCIS on the slide. We noted if a dense chronic inflammatory infiltrate surrounded DCIS.

Results: The mean age of our 46 pts was 64. Oncotype score was low in 33 pts (72%) of whom 6 recieved RT. Oncotype was intermediate in 8 pts (17%) of whom 6 recieved RT. Oncoytpe was high in 4 pts (9%) of whom 2 recieved RT, one refused RT and one has no follow-up. In one pt there was a test failure. PR via immunohistochemistry was positive in 40 pts (87%) negative in 4 (9%) and unknown in 1 (5%). In 24 pts PR was $\geq$ 90% and 22 of them (92%) had a low score. PR$\geq$90% in DCIS was significantly associated with a low score (p=0.0051). The H&E slide of the block sent for Oncotype was available in 34 pts. The number of mitotic figures in all DCIS glands was 0 in 19 pts of whom 16 (84%) had a low score. The number of mitotic figures was $\geq$ 1 (range 1 to 8) in 15 pts of whom 7 (47%) had a low score. A mitotic count of 0 for DCIS was significantly associated with a low score (p=0.03). A dense chronic inflammatory infiltrate surrounded DCIS in 4 pts and 2 of these pts had a high score (p=0.047). Twelve pts had PR$\geq$90%, 0 mitotic figures and were without dense chronic inflammation around DCIS and all had a low score. 5 pts had a combination of atleast two of the following - negative PR, mitotic count$\geq$1 and / or dense chronic inflammation around DCIS and none had a low score.

Conclusions: DCIS with PR $\geq$90%, no mitotic figures and absence of dense chronic inflammation around DCIS, as seen in 28% of pts, had a low score in 100% of cases. Conversly none of the 11% of pts with a combination of atleast two of the follwoing :

negative PR, mitotic count ≥1 and/or dense chronic inflammation around DCIS had a low score. These histopatholgic features in DCIS might be useful surrogate for Oncotype score. IT also suggests that mitotic counting in DCIS might be prognostic.

167 Tumor Infiltrating Lymphocytes and Response To Neoadjuvant Chemotherapy in Invasive Breast Cancer

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Background: Neoadjuvant chemotherapy (NAC) is often used to treat invasive breast cancer (IBC). A pathologic complete response (pCr) (RCB-0) or minimal residual disease (RCB-1) is associated with the best prognosis. The immune system appears to play a role in response to NAC and a signficant tumor lymphocytic infiltrate (TIL) may improve response rate. We examined pre chemotherapy(pch) core biopsies (bxs) and correlated TIL with response to NAC.

Design: We evaluated pch core bxs in 95 pts with IBC. We visually estimated the % of mononuclear cells both in direct contact with tumor cells and within tumor stroma. We recorded ER, Her2/Neu and the mitotic count score (Nottingham Score). We used pathology reports from post chemotherapy resections to calculate Residual Cancer Burden (RCB) (www.mdanderson.org/.../calculatore-rcb-pathology). We considered RCB-0 (pCr) and RCB-1(minimal residual disease) to represent excellent response to NAC.

Results: We evaluated pch core bxs from 95 pts with IBC who received NAC. Post chemo resections demonstarted RCB-0 in 21 pts (22%), RCB-1 in 15 pts (16%) RCB-2 in 25 pts (26%) and RCB -3 in 34 pts (35%). The percentage of TIL in pch core bxs , in either tumor stroma or epithelium was ≥ 50% in 18 pts, <50% and ≥40% in 6 pts, <40% and ≥30% in 5 pts and ≤20% in 66 pts. Of the 24 pts with TIL ≥ 40% , 16 (67%) had RCB-0 or 1. Of the 71 pts with TIL <40%, 20 (28%) had RCB 0 or 1. There was a statistically significant association between TIL ≥ 40% and RCB-0 and 1 (p=.0013). Her2/Neu was + in 31 pts and 16 (52%) had RCB 0 or 1. Her2/Neu was (-) in 62 pts and 20 (32%) had RCB 0 or 1. The association between Her2Neu positivity and RCB-1 or 0 was not statistically significant (p=0.113). ER was + in 44 pts and (-) in 51 pts. 24 of 51 (47%) ER (-) pts had RCB 0 or 1 and 12 of 44 (27%) ER+ had RCB 0 or 1; this difference was not statistically significant (p=0.06). 34 tumors were triple negative and 14 (41%) had RCB-0 or 1 (p=0.06). Mitotic count score was 3 in 29 pts and less than 3 in 59 pts, and could not be determined in 7 pts. RCB was 0 or 1 in 10 of 29 (34%) pts with mitotic score 3 and RCB 0 or 1 in 25 of 59 (42%) of pts with mitotic score < 3 (p=0.50).

Conclusions: We evaluated ER, Her2/Neu , mitotic score and TIL and correlated with response to NAC. Only TIL 40% or greater in pch core bxs was significantly associated with pCr or minimal residual disease . Quantification of the % TIL can easily be done on H and E slides and may be important in the evaluation of IBC when NAC is considered.

168 Correlation of Protein Expression With Chromosomal Copy Number Alterations for Risk Classification of Ductal Carcinoma In Situ

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Background: The diagnosis of ductal carcinoma *in situ* (DCIS) is rising in number due mammographic screenings, contributing to concerns of over-diagnosis and over-treatment. We aimed to determine the correlation of protein expression with chromosomal copy number alterations (CNAs) as part of an effective risk-stratification for identifying high-risk DCIS with the ultimate goal of improved personalized management for early breast cancer.

Design: A tissue microarray (TMA) of samples containing DCIS only versus DCIS with concurrent invasive breast cancer (IBC) was used to assess the correlation of CNAs of 8q24 (BAC clone RP11-1136L8) and 11q13 (BAC clone CTD-2537F6) by fluorescence in situ hybridization (FISH), reported by The Cancer Genome Atlas to be among the most frequent CNAs in IBC, with protein expression of C-MYC on 8q24 (Leica 1472-1) and BCL-1 on 11q13 (Ventana RM-9104-S) by immunohistochemistry (IHC) in the DCIS component of the biopsies.

Results: We studied 199 samples diagnosed as DCIS only or DCIS with IBC for IHC and FISH correlation for C-MYC expression and 175 for BCL-1 expression. We found that the number of C-MYC expression positive cases were similar between DCIS only or DCIS with IBC (71 positive of 107 total DCIS versus 67 positive of 92 total DCIS with IBC). However, when we stratified the cases by C-MYC amplification, the presence of the amplification in DCIS with IBC was associated with more than half of the cases with positive protein expression with a sensitivity of 61.2% (41/67) when compared to DCIS alone at 38.0% (27/71), and these differences were statistically significant (*P*=0.0104 by Fisher's exact test). Many fewer cases of DCIS were amplified for BCL-1 or had BCL-1 expression; however a similar trend was observed where CNAs in DCIS with IBC were more predictive of IHC positivity with a sensitivity of 75.0% (6/8) when compared to DCIS alone at 53.9% (7/13) (*P*=0.3999 by Fisher's exact test).

Conclusions: There is an increased need for risk-adapted management of DCIS and early breast neoplasia. This study demonstrates the potential for combined protein expression and DNA copy number studies for improving the classification of high-risk DCIS.

169 Use of 2013 ASCO-CAP Breast Carcinoma HER2 Scoring Guidelines Results in Shifts of Scoring Categories With Slight Change in IHC-FISH Concordance

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Background: In 2013, revised ASCO-CAP guidelines for immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) HER2 testing and scoring of breast cancers were released. These included changes in the definitions of HER2 1+, 2+, and 3+ IHC scoring. We wished to investigate the effects of the new scoring definitions on the distribution of HER2 IHC scores and the impact on IHC-FISH concordance. Our hypothesis was that the new definitions would lead to increased numbers of 1+ and 2+ IHC cases with minimal impact on IHC-FISH concordance.

Design: The results of scoring of all breast cancer specimens received for HER2 IHC and/or FISH testing were tabulated during two parallel time intervals: January-August 2013 (pre-2013 ASCO-CAP) and January-August 2014 (post-ASCO-CAP). A total of 1,813 breast cancer cases received from a wide range of laboratories around the United States had been submitted to PhenoPath for HER2 assessment in the 2013 time period, and 1,447 in 2014. On all cases submitted for primary FISH analysis, nonreported HER2 IHC studies were also performed for quality assurance purposes. All IHC was performed using the SP3 rabbit monoclonal anti-HER2 antibody on a Dako autostainer. All FISH was performed using the PathVysion dual probe kit, assisted by image analysis using the Metasystems Metafer 4 software.

Results: Comparing the 2014 to the 2013 IHC data, the percentage of cases scored as 0 decreased from 29% to 21%; the percentage of cases scored as 1+ remained nearly the same (40% v. 42%); the percentage of 2+ cases increased from 23% to 29%, and the percentage of 3+ cases increased slightly from 7.6 to 8.6%. Amongst the IHC 2+ (equivocal) cases, analysis of corresponding FISH results in the 2013 and 2014 revealed that the percent of FISH negative cases increased from 79% to 87%, and the percent FISH positive (³2.0 ratio) decreased from 12% to 9.0%. Among the cases sent in for primary HER2 FISH, the 2013 positive and negative concordance rates for FISH-IHC were 95.7% and 99.7%, respectively; these concordance rates in 2014 were 97.3% and 99.3%.

Conclusions: The data support our hypothesis that a principal effect of the 2013 ASCO-CAP HER2 scoring guidelines has been a shift in the 'binning' of cases, with a significant number of cases previously scored as 0 shifting into the 1+ category, and cases previously scored as 1+ into the 2+ category. Overall, a slight increase in positive IHC-FISH concordance was noted (95.7% to 97.3%). 2013 ASCO-CAP Guidelines may result in increased overall testing costs owing to these category shifts.

170 Genetic Events in the Progression of Adenoid Cystic Carcinomas of the Breast To High-Grade Triple-Negative Breast Cancers

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Background: Adenoid cystic carcinoma (AdCC) of the breast is a rare triple-negative breast cancer (TNBC) harboring the recurrent *MYB-NFIB* fusion gene. The phenomenon of high-grade (HG) transformation of AdCC and its genetic basis has not been investigated in AdCCs of the breast. Here, we sought to define the repertoire of somatic genetic alterations involved in the progression from AdCC to HG-TNBC.

Design: Two breast AdCCs associated with HG-TNBC of no special type (NST) were centrally reviewed by three pathologists. Fluorescence *in situ* hybridization and RT-PCR were employed to detect the *MYB-NFIB* fusion gene. DNA samples from each microdissected tumor component and matched normal tissue were subjected to massively parallel sequencing targeting all exons of 490 genes, including the most frequently mutated in breast cancer and actionable cancer genes. Somatic single nucleotide variants were detected by MuTect, insertion and deletions by Varscan2 and Strelka. All mutations were confirmed by Sanger sequencing. Gene copy number profiling was performed using Affymetrix OncoScan arrays.

Results: The *MYB-NFIB* fusion gene and its transcript were detected in all components from each case. Moreover, the conventional AdCC and the HG-TNBC of case 1 shared identical somatic mutations in *CHD6*, *CTNNB1*, *RPS6KB2* and *EP300*, whereas mutations in *SF3B1* and *NOTCH1* were only found in the latter. In case 2, in addition to the *MYB-NFIB* fusion gene, a *KMT2C* somatic mutation was present in all components analyzed, whereas mutations of *MYB*, *CDK12* and *STAG2* were found only in the HG-TNBC. Gene copy number profiling revealed shared genetic alterations between the AdCC and the HG-TNBC components, but the latter displayed increased genomic complexity in both cases.

Conclusions: The progression from AdCC to HG-TNBC of NST involves the acquisition of additional genetic alterations. We also documented a subset of HG-TNBC of NST harboring the *MYB-NFIB* fusion gene, the second recurrent fusion gene identified in TNBCs.

171 Differential Expression of miR-139 in Breast Cancer

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Background: The discovery of microRNAs (miRNAs) has created new opportunities to better understand the complexity of cancer. miRNAs are non-protein coding genes that regulate gene expression at the translational level. They play a key role in oncogenesis, tumor progression and metastasis. miRNAs have the potential to be oncogenes or tumor suppressors in a given cellular context. While the overexpression of certain miRNAs has been shown to promote metastasis, the overexpression of others correlated with reduced metastatic activity. A better ability to assess the risk of recurrence in breast cancer patients would greatly facilitate clinical decisions in surgical and adjuvant treatment options for breast cancer patients. The development of reliable risk assessment biomarkers will empower oncologists to tailor treatment options that are more effective with less undesirable side effects. Based on several studies demonstrating the role of microRNA miR-139-5p (miR-139), we **hypothesize** that miR-139 might play a key role in tumor progression and metastasis in breast cancer.

Design: miR-139 expression was correlated with tumor grade, type, ER, PR, Ki67 and HER2 status, tumor size, lymph node status, patient's age and overall survival in 74

invasive ductal carcinomas. RNA samples were isolated from the FFPE samples using miRNeasy FFPE kit (Qiagen) per manufacturer's instructions. Concentration of RNA was detected by NanoQuant Infinity Pro200 (Tecan). The miR139-5p specific primers and the SNORD 95 primers were used for RT-PCR in this study.
Results: miR-139 expression is associated with aggressive pathologic and clinical features. miR-139 is down regulated in higher grade (grade I vs. grade II, p<0.001, grade II Vs. grade III, p=0.022 and grade I vs. grade III, p<0.001), ER negative (p=0.0008), PR negative (p=0.005), HER2+ (p=0.0537) and highly proliferative tumors (Ki67>15%, p=0.0002). Its reduced expression is not associated with tumor size (>2 cm, p=0.0708) or patient's age (>50 years, p=0.434). When its levels of expression was correlated with local metastasis and overall survival, there was no significant difference in its expression in tumors with nodal status (p=0.264). In contrast deceased patients had significantly lower levels compared to alive patients (p=0.027).
Conclusions: miR-139 is differentially expressed in breast carcinomas. Its expression is significantly associated with several clinicopathological parameters and is associated with aggressive tumor behavior and disease progression. Modulation of miRNA expression may serve as an attractive approach for treatment of aggressive and advanced breast carcinomas.

172 Glucocorticoid Receptor Expression in Triple Negative Invasive Ductal Carcinoma of the Breast: A Potential Therapeutic Target

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Background: Breast cancers which express the estrogen receptor (ER), progesterone receptor (PR), or overexpress HER2 provide actionable therapeutic targets. However, 15-20% of breast cancers are triple negative (TNBC), lacking a therapeutic target, and are associated with a worse prognosis. Activation of the glucocorticoid receptor (GR) has diverse effects depending on cell type. In ER- breast cancer, activation has been demonstrated to inhibit apoptosis of tumor cells, whereas its antagonism is associated with increased tumor cell death. Therefore, GR represents a potential therapeutic target. The current study was undertaken to investigate GR expression and its relation to other pathologic characteristics in previously diagnosed invasive ductal carcinoma (IDC).
Design: Consecutive cases of IDC from 2013 with sufficient material in the biopsy specimen for further testing were identified. Immunohistochemistry for GR was performed and results correlated with H&E findings, ER/PR/HER2 status, and demographic data. GR as well as ER and PR status were analyzed as binary results (positive designated as ³1% expression for ER, PR, and GR) and as Allred scores. Data were examined using Student's t-test.
Results: A total of 95 cases of IDC (61% African American, 29% non-Hispanic Caucasian, 7% Asian, 3% Hispanic) were analyzed: 12% were grade I, 32% were grade II, and 56% were grade III. ER was expressed in 63% (100% grade I, 83% grade II, 46% grade III), HER2 was overexpressed in 16% (0% grade I, 27% grade II, 13% grade III); 32% were TNBC (0% grade I, 13% grade II, 87% grade III; overall 0% of grade I are TNBC, 13% of grade II are TNBC, 48% of grade III are TNBC). GR was expressed by 88% of IDCs: 93% grade I, 80% grade II, and 82% grade III. 93% of TNBCs and 94% of ER- IDCs expressed GR. Additionally, ER- IDCs had higher GR Allred scores than ER+ IDCs (6.66 vs. 5.88, p=0.03). Age also predicted GR status: women <45 years old were more likely to express GR than women ≥45 (100% vs 87.5%, p<0.01) and to have higher Allred scores (6.78 vs. 6.10, p=0.01).
Conclusions: The vast majority of our cases of IDC were found to express GR. 93% of TNBC and 94% of ER- IDCs expressed GR, with stronger expression of GR identified in ER- IDCs as compared to ER+ IDCs. Additionally, women <45 years of age have higher expression of GR and would likely derive the most benefit from its antagonism. GR antagonism represents a potential therapeutic target, particularly in TNBC.

173 Evaluation of the Benefit of Additional Excision in Cases of Papillomas of the Breast Diagnosed on Core Biopsy – Should a Papilloma Be Excised or Not? A Single Institution Experience

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Background: Papilloma of the breast is a common finding on core biopsies that can often account for a mass seen during the patient's imaging studies. Although a benign diagnosis, the patient often undergoes subsequent excision. In our institution, the decision to undergo excision was made variably but more commonly in patients whose clinical suspicion remained elevated for a higher risk lesion, or in those who had the lesion removed for symptom relief and psychological comfort. The objective of this study is to evaluate how many patients had excision of a papilloma diagnosed on core biopsy without atypia, and how many of these patients benefitted from excision because of identification of a more significant lesion in the excision, which would have otherwise not been found.
Design: 150 cases of breast core biopsies diagnosed as papilloma without atypia were identified in the surgical pathology archive from 2008 to 2014. It was documented if the patient then had a subsequent procedure (i.e. excisional biopsy or lumpectomy) or if no further intervention was performed. The results of the subsequent excision were evaluated.
Results: 79 of the 150 (53%) patients did not have any additional procedures, while the remaining 71 (47%) had a subsequent excision. Of those with additional excisions, 8 (11%) were found to have a higher risk lesion. These lesions included 3 (4%) cases of ductal carcinoma in situ (DCIS), 3 (4%) cases of atypical ductal hyperplasia, 1 (1.5%) case of lobular carcinoma in situ and 1 case (1.5 %) of atypical lobular hyperplasia. Only 2 of 3 of the cases of DCIS (3% of 71 excised cases) involved the papilloma while in the remaining 6 cases (8%) the more significant lesions were found in surrounding breast tissue.
Conclusions: There is lack of consensus regarding excision of papillomas diagnosed on core biopsy. Less than 50% of papillomas diagnosed on core biopsy at our institution were excised. Of those excised, the overwhelming majority remained benign but 11% were found to have a more significant lesion. Overall, the incidence of the papilloma harboring a more significant lesion is only 3%. It is important for patient management to be aware of the small risk of finding a more high risk lesion in the subsequent excision for the papilloma. The majority of these excisions may be unnecessary and an effort to eliminate extra surgery based on radiologic and clinical risk factor stratification along with proper patient consulting could further benefit patients.

174 Phosphohistone H3 Expression Correlates With Manual Mitotic Counts and Aids in Identification of "Hot Spots" in Fibroepithelial Tumors of the Breast

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Background: Classification of fibroepithelial tumors (FETs) of the breast relies heavily on assessment of mitotic activity, among other histopathologic parameters. Routine H&E mitotic counts can be subjective and time-consuming. Difficulty may arise in distinguishing mitoses from apoptotic cells and identifying mitoses in a "condensed" state, as can occur with hypoxia and suboptimal fixation. Phosphorylation of histone H3 protein (PPH3) is tightly correlated with mitotic chromatin condensation. PPH3 has been used as an immunohistochemical (IHC) marker of mitoses in multiple tumors/sites. In this study, we examined the utility of PPH3 in assessing proliferative activity of FETs and compared PPH3 with H&E-determined mitotic counts.
Design: 108 FETs including 27 fibroadenomas (FA), 30 benign phyllodes tumors (BPT), 28 borderline phyllodes tumors (BlnPT), and 23 malignant phyllodes tumors (MPT) from 98 patients were identified. Slides were reviewed to confirm the diagnoses. PPH3 staining was performed on 1 unstained paraffin section from each tumor. Mitoses were counted on H&E and PPH3-stained slides in 20 high-power fields (HPFs) (HPF=0.24 mm^2), beginning in areas dense in PPH3-stained objects seen at 10x magnification. Statistical analysis was performed.
Results: PPH3-stained mitoses were readily identifiable at 10x magnification and allowed rapid identification of mitotic "hot spots." Median mitotic counts/10 HPFs for FA, BPT, BlnPT, MPT were 0.05, 0.74, 4.71, and 13.65, respectively on H&E, and 0.18, 0.78, 5.09, and 16.05, respectively for PPH3. There was a strong positive correlation between H&E and PPH3-determined mitotic counts (r=0.91, P<.001). Both H&E and PPH3-determined mitotic counts were significantly different between tumor grades (P<.001). Based on PPH3-determined mitotic counts, 2 cases would be reclassified, both from BlnPT to MPT.
Conclusions: PPH3 mitotic counts correlate with manual H&E counts in FETs. While H&E-determined manual counting remains the gold-standard for assessing mitotic activity in FETs, PPH3 may be helpful as an adjunctive tool in this setting, specifically for identifying mitotic "hot spots." Using PPH3, a small number of cases were reclassified from BlnPT to MPT, for which treatment is similar. Additional study is needed to determine the prognostic significance of reclassifying FETs using PPH3.

175 Stability of Estrogen Receptor (ER), Progesterone Receptor (PR), Ki-67 and HER2 Status in Breast Carcinomas After Neoadjuvant Chemotherapy (NAC)

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Background: NAC is used in the treatment of breast carcinoma to reduce tumor burden. Currently, there are conflicting data regarding stability of biomarker status after NAC. Significant change in biomarker status may affect the post-surgical utility of hormone-directed or HER2-specific therapy. We sought to evaluate this issue at our institution.
Design: 47 cases of invasive breast carcinoma with incomplete response to NAC and available pre- and post-NAC tissue samples were identified. Immunohistochemistry was performed for ER, PR, Ki-67 and HER2 on tissue microarray or whole tissue slides. For ER and PR, ³1% nuclear positivity with moderate or strong staining intensity was positive. A case was weakly positive if ³1% but £10% nuclear positivity with weak staining intensity was seen. Ki-67 was "low" if <15% and "high" if ≥ 15% of nuclei stained positive. Pattern and intensity of membrane staining of HER2 was evaluated as: 0 =£10% faint staining, 1+=faint staining in >10%, 2+=weak to moderate staining in >10% or strong circumferential in £10%, 3+=strong complete staining in >10% with interpretation as: 0 and 1+=negative, 2+=equivocal, 3+=positive.
Results: A majority of cases showed no change in ER (83%), PR (81%) and HER2 (96%) status. ER changed from negative to positive in 5/47 (11%), all of which were weakly positive. Conversely, 3/47 (6%) changed from a positive to negative; only 1 of which changed from weakly positive to negative. For PR, 5 cases changed from a negative to positive change (11%), one of which had the same change in ER and 2 of which only changed to weakly positive. Conversely, 3/47 (6%) changed from positive to negative, none of which also had the same change in ER. For HER2, 1/47 (2%) changed from negative to positive, confirmed to be amplified by HER2 FISH, while none changed from positive to negative. While in most cases (66%) there was no change in Ki-67 expression, a decrease was seen most often in cases that changed (13/16; 81%).
Conclusions: Contrary to the reported trend of decreasing ER and PR expression after NAC, we found a change from negative to positive was more common, possibly due to the inclusion of weakly positive cases, leading to a possible role for hormonal therapy in these patients. A positive change in HER2 was rare which is consistent with the reported finding of HER2 stability after NAC. Ki-67 tendency to decrease after NAC is also in line with published findings and therefore, best assessed in the pre-NAC sample.

176 Prognostic Impact of DLC1 in ER-Positive Breast Cancer

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Background: Deleted in Liver Cancer-1 (DLC1), a Rho-GTPase-activating protein, is down-regulated in a number of solid and hematological cancers. *DLC1* can act as tumor and metastasis suppressor. Restoration of *DLC1* expression has been shown to suppress tumorigenicity as well pulmonary metastasis in preclinical models. However, its clinical relevance is not clearly delineated in breast cancer.

Design: To determine the clinical significance of *DLC1* downregulation in breast cancer, we evaluated its expression in a cohort of 60 paraffin-embedded ER-positive, node-negative breast carcinomas with low, intermediate, and high Onco*type* DX recurrence scores (19, 21, and 20 cases, respectively) using a real-time quantitative Reverse Transcription PCR (qRT-PCR). To further confirm the clinical relevance, expression levels and prognostic values were also assessed in large cohorts using publicly available Affymetrix gene expression datasets.

Results: Quantitative RT-PCR analysis showed that *DLC1* expression was significantly lower in ER+ node-negative patients with Onco*type* DX high recurrence score when compared with low recurrence score patients (Fold-change: 47% down; *P*=0.02). In publicly available Affymetrix breast cancer datasets, patients with ER-positive tumors having high *DLC1* levels were significantly more likely to have improved relapse-free survival (RFS; *P*=0.002), distant metastasis-free survival (DMFS; *P*=0.003) and overall survival (OS; *P*=0.003) compared to those with low and moderate levels. In multivariable analysis, *DLC1* remained a significant prognostic parameter

Table 1. Multivariable Analysis in ER+ Patients for Relapse-Free Survival and Overall Survival (Affymetrix datasets)

	RFS		OS	
Variable	HR (95% CI)	P value*	HR (95% CI)	P value*
Moderate vs high DLC1	1.41 (1.03-1.91)	0.029	1.45 (0.99-2.12)	0.05
Low vs high DLC1	1.44 (1.01-2.04)	0.043	1.55 (1.03-2.33)	0.03
LN+ vs LN-	1.43 (1.08-1.92)	0.015	2.17 (1.56-3.03)	4.77E-06
Grade 3 vs 1 + 2	1.24 (0.91-1.68)	0.18	1.44 (1-2.07)	0.05
Pre vs Post	1.23 (0.92-1.64)	0.16	0.63 (0.44-0.90)	0.012
T2 vs T1	1.94 (1.47-2.57)	3.52E-06	2.02 (1.44-2.84)	5.20E-05

A prognostic impact was not observed in ER-negative tumors.

Conclusions: The expression levels of *DLC1* in ER-positive breast cancer are associated with both relapse-free and overall survival. These findings suggest that development of strategies for therapeutic restoration of *DLC1* could have therapeutic benefit.

177 Triple Negative Breast Cancer: Nuclear or Cytopasmic FOX1? Location, Location, Location

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Background: Aberrant protein expression is described in malignat and non-malignat conditions. The aberrant expression may be either nuclear or cytoplasmic. Nuclear-cytoplasmic transport is an important aspect of normal cells function. Forkhead-box (FOX) family proteins are DNA binding proteins regulating transcription and DNA repair and normally expressed in the nucleus. FOX1 functions as tissue specific alternative splicing (AS) regulator, stimulates expression of genes involved in cell division, attenuation of oxidative stress and tumor metastasis. FOX1 depletion causes cell death by mitotic catastrophe, often due to aberrant G2 checkpoint control. The aim of the study was to assess the nuclear vs. cytoplasmic immunohistochemical (IHC) expression of FOX1 in human breast cancer in correlation with hormone receptors and HER overexpression status.

Design: Invasive mammary carcinomas were grouped into triple negative tumors (TNT) and non-TNT. The non-TNT carcinomas included estrogen and/or progesterone receptor and/or Her2 overexpessing tumors. Tissue microarrays were stained with FOX1 monoclonal antibody. Nuclear staining in more than 5% of cells was considered positive. Cytoplasmic staining was considered positve if more then 3+ in 5% of cells or more then 2+ in 10% of cellls. Age, race, tumor grade and stage were collected and analyzed using Wilcoxon rank sum test for numerical variables and chi-square or Fisher's exact test for categorical variables where appropriate.

Results: A total of 388 breast cancer patients were evaluated: 183 were TNT and 205 were non-TNT tumors. The age at diagnosis ranged from 24-90 years of age. TNT patients were significantly younger at the time of diagnosis (p=0.022), with the median age at diagnosis 54 years of age vs. 57 in non-TNT. Nuclear FOX-1 stainin is significantly higher in non-TNT tumors compared to TNT (<0.001), with FOX1 expressed in 178 non-TNT tumors but only in 19 TNT. Cytoplasmic staing was strinkigly more frequent in TNT 59 (49.5%) compared to 79 (38.79%), p<0.01. Race, tumor grade, stage, or age at diagnosis also did not affect FOX1 expression.

Conclusions: 1. Cytoplasmic aberrant FOX-1 expression may differentiate breast cancer tumor subtype and explain the more aggressive nature of TNT. Furthemore it may be associated with carcinogenisis and tumor progession in younger women.

2. Targeted inhibition of FOX-1 protein cytoplasmic transport may represent a powerful theranostic agent in the managemnt of a subtype of breast carcinomas with no current targeted therapy.

178 Massively Parallel Sequencing Analysis of Acinic Cell Carcinoma of the Breast

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Background: Acinic cell carcinoma (AciCC) is a rare salivary gland-type tumor of the breast, displaying serous acinar differentiation. Despite its triple-negative phenotype, AciCCs are reported to have an indolent clinical behavior. We sought to investigate whether breast AciCCs would be underpinned by genetic alterations found in 254 breast cancer-related genes, and to define whether AciCCs would have a mutational repertoire distinct from that of other triple-negative breast cancers (TNBCs).

Design: Three pure and seven mixed breast AciCCs (six AciCC-invasive ductal carcinomas of no special type and one AciCC-metaplastic carcinoma) were included in this study. DNA was extracted from microdissected formalin-fixed paraffin-embedded sections of tumor and normal tissue. In mixed cases each component was microdissected separately. DNA of sufficient quantity was obtained from 2 pure AciCCs, from the AciCC component of 7 mixed cases and from the non-AciCC components of 4 cases. Massively parallel capture sequencing targeting all exons of the 254 genes most frequently mutated in breast cancer was performed. Single nucleotide variants, and insertions and deletions were detected by MuTect, Strelka and VarScan2. Copy number alterations (CNAs) were identified using VarScan2 and GISTIC2.0.

Results: Two pure AciCCs and AciCC components from five mixed cases harbored somatic *TP53* mutations. One of the *TP53* wild-type cases carried a mutation and loss of heterozygosity of *MLH1*, in addition to somatic mutations in *RB1, NF2* and *ATR.* Additional somatic mutations affecting breast cancer-related genes found in AciCCs included *PIK3CA*, *MTOR*, *CTNNB1*, *BRCA1*, *ERBB4*, *ERBB3, INPP4B* and *FGFR2*. CNA analysis revealed complex patterns of gains and losses similar to those of TNBCs. Of the 4 mixed cases analyzed, identical somatic mutations were found in the AciCC and non-AciCC component of 2 cases (4 and 7 mutations in common in each case), however additional somatic mutations were identified in the non-AciCC component. In the mixed cases lacking somatic mutations in common, similar patterns of gene CNAs were found in both components of one case.

Conclusions: Breast AciCCs display genomic features similar to those reported in TNBCs, harboring complex CNA profiles and *TP53* mutations as the most common genetic event. AciCCs may constitute the substrate for the development of more aggressive forms of TNBCs.

179 Massively Parallel Sequencing Reveals That Microglandular Adenosis Is a Clonal Neoplastic Lesion of Triple-Negative Phenotype

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Background: Microglandular adenosis (MGA) is a lesion composed of small infiltrating glands lined by S100-positive, estrogen receptor (ER)-negative epithelial cells and lacking a myoepithelial cell layer. Although classified as a benign epithelial proliferation, there is evidence to suggest that MGA may constitute a non-obligate precursor of triple-negative breast cancer (TNBC). We sought to define the mutational landscape of MGAs and of TNBCs arising in MGA, and to determine whether MGAs may constitute the substrate from which the TNBCs originated.

Design: Six cases of MGAs and three of atypical MGAs (AMGAs) associated with *in situ* or invasive TNBC were collected. DNA from distinct morphologic components and matched normal tissue was extracted from microdissected representative sections and subjected to massively parallel sequencing targeting all coding regions of 273 genes recurrently mutated in breast cancer or related to DNA repair. Single nucleotide variants were detected by MuTect; insertions and deletions were identified by Strelka and VarScan2.

Results: MGAs (n=6) and AMGAs (n=3) consistently displayed at least one somatic mutation (range 5-17 and 1-10, respectively), whereas TNBCs (n=4) associated with MGA/AMGA harbored 6 to 11 somatic mutations. Four to 7 mutations identified in MGAs/AMGAs were also detected in their associated invasive TNBCs, and in all cases identical *TP53* mutations were found in the MGA and/or AMGA and in the associated TNBC; however the latter harbored additional mutations affecting known cancer genes. In the MGAs/AMGAs lacking *TP53* mutations, mutations affecting known driver genes, such as *PIK3CA, ERBB3*, *PTEN*, *FGFR2* and *INPP4B,* were identified.

Conclusions: MGA is a clonal and neoplastic lesion, harboring recurrent mutations in *TP53* and other *bona fide* cancer genes. Identical somatic mutations were identified in MGAs/AMGAs and matched invasive TNBCs, providing evidence to suggest that these lesions may constitute non-obligate precursors of TNBCs.

180 Characterization of Molecular Alterations and Clinical Utilization of Targeted Next-Generation Sequencing in Advanced Breast Cancer

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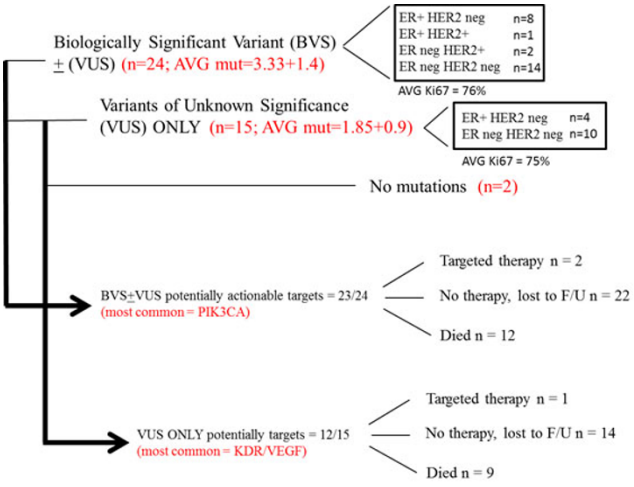
Background: Women with aggressive, often widely metastatic breast cancers are unresponsive to current chemotherapy, with dim prospects and few therapeutic options. Recent advances in genomic profiling represent a new paradigm of cancer classification, with potential to match patients with targeted therapies.

Design: A search of clinical data at our institution yielded 41 advanced breast cancer patients with avg age of 54 (range 31-75). The cancer samples underwent next generation

sequencing via Ion Torrent PGM. Specimen characteristics were as follows: 4 (10%) cytology and 37 (90%) surgical specimens; 33 (80%) metastatic and 8 (20%) primary/recurrent carcinomas; 26 (63%) in-house and 15 (37%) outside/referral. A standard 46 or 50 targetable cancer gene panel with approximately 2,800 mutations was reported.
Results: Among 41 patients, 2 (5%) showed no genetic alterations, 15 (36%) showed variants of unclear significance (VUS) only and 24 (59%) showed at least 1 biologically significant variant (BSV)+/-VUS. The samples with VUS only showed an average of 1.85±0.9 alterations and 8/15 (53%) were metastases. The samples with BSV showed a significantly more alterations (3.33±1.4, *p≤0.01) and larger fraction were metastases (23/24, 96% *p≤0.01). Overall, 110 aberrations were observed in 15 of 50 targeted genes. Every patient had a unique molecular profile. BSV seen in ≤2 patients within the cohort included genes: TP53 (n=13), PIK3CA (n=13), KRAS (n=3), PTEN, APC, STK11, ERBB4 (n=2). BSV aberrations included 36 point mutations (80%), 3 amplifications (7%), 3 deletions (7%), 2 insertions (4%) and 1 frameshift (2%). The tested samples also showed very high Ki67 index (avg 75%) and were largely (24/39, 62%) hormone receptor and HER2 negative. All of the NGS reports were promptly reviewed (95% within 1 day). Among patients with BSV, 2 of 23 (9%) received targeted therapy.
Conclusions: Advanced breast cancers show a patient-unique genetic landscape, though with several recurrent alterations. The majority of tested samples were metastases and both hormone receptor and HER2 negative. From clinical standpoint, NGS reports are promptly reviewed but utilization of targeted therapy is limited.



181 Comparison of Invasive Pleomorphic Lobular Carcinoma With Invasive Classic Lobular Carcinoma: Clinicopathologic and Biomarker Analysis

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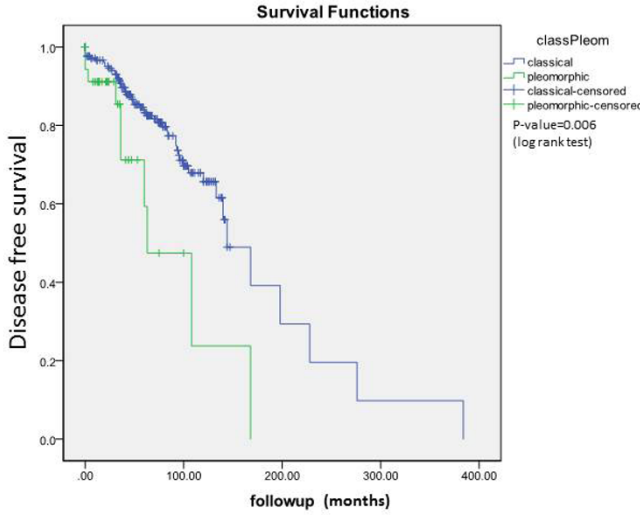
Background: Pleomorphic invasive lobular carcinoma (PILC) is a rare, aggressive and distinct morphological variant of invasive lobular carcinoma (ILC) characterized by nuclear atypia and pleomorphism compared to classic ILC (CILC). The aim of this study was to evaluate the clinicopathologic characteristics, tumor biomarkers and prognosis of PILC compared with CILC.
Design: We retrospectively reviewed the medical records of 255 patients with ILC and compared the clinicopathologic parameters and Immunohistochemical (IHC) stains for ER, PR, MIB-1 and FISH for HER2 and disease free survival (DFS) of patients with PILC from CILC.
Results: Of the 255 cases, 40 cases were PILC and 215 CILC. Seventy six (76/184,41.32%) cases showed axillary lymph node metastases. Our results showed that compared with classic ILC, PILC had a higher T stage, (P < .0001) and larger tumor size. Nodal involvement, lymphovascular invasion, and metastasis of CILC were less frequent compared to PILC with p-value of 0.001, 0.01 and 0.014 respectively.PILC was more commonly positive for HER2(P < .025), and MIB-1(P<.047) while negative for ER and PR compared to CILC. The patients with CILC had better disease free survival compared to PILC (p=0.006, log rank test).



When divided into different strata/catergories, ER positive and PR negative CILC cases showed better DFS than ER positive and PR negative PILC cases(p=0.045,and p=0.001 log rank test)respectively. Similarly, CILC cases in patients >50 years showed better DFS than patients >50 years with PILC (p=0.0001, log rank test).
Conclusions: Classical invasive lobular carcinoma is characterized by less nodal involvement, lymphovascular invasion, smaller tumor size, lower metastatic propensity and better disease free survival than PILC. When divided into different strata, ER positive, PR negative and >50 years patient groups with CILC show even significant better DFS than PILC. PILC exhibit an adverse biomarker profile (positive HER2 neu, negative ER and PR and high Ki-67) as compared with classic ILC. These findings suggest that there is biological significance in differentiating PILC and CILC and that this may enhance tumor aggressiveness in certain strata of ILC.

182 Quality Assurance in Breast Pathology: Lessons Learned From a Review of Revised Reports

Beth Harrison, Deborah Dillon, Andrea Richardson, Jane Brock, Anthony Guidi, Susan Lester. Brigham & Women's Hospital, Boston, MA.

Background: Breast is the organ site most frequently associated with surgical pathology report defects. Few studies have investigated the types of error and the mechanisms of error detection specific to these diagnostically challenging specimens.
Design: We performed a retrospective study of all revised pathology reports for breast surgical specimens at a large academic medical center over a 5 year period from 2009 to 2013. Reasons for revising a report were classified by utilizing published taxonomies. Special emphasis was placed on characterizing major and minor diagnostic errors.
Results: Over the 5 year period, 1% of breast pathology reports were revised (119 of 12,228). The majority of revisions (74%) were due to non-diagnostic issues including changes in specimen identification or laterality (10%), patient registration (14%), or typographical errors (29%). A minority (26%) involved changes in diagnosis. There were 14 major changes that could have had an effect on clinical care and 13 minor changes that were unlikely to alter care. Most of the major changes (9 cases) involved classification of cancer as DCIS or invasive carcinoma. Two involved revision of a diagnosis of DCIS to ADH, 1 misdiagnosis of lymphoma as carcinoma, 1 identification of a lymph node metastasis, and 1 failure to recognize amyloid. Of note, a majority (10 of 14) of the amendments for major changes were discovered during review of immunohistochemical (IHC) studies for receptors, either due to review by a second pathologist or to more obvious diagnostic findings revealed on the deeper levels prepared for the study. Two cases were revised after subsequent surgery revealed more diagnostic findings. The final two cases were revised after the original pathologist requested a second opinion or obtained additional IHC studies.
Conclusions: A review of revised reports provides valuable information for quality assurance and improvement within a pathology department. In our practice setting, IHC studies for predictive factors are often reported by a pathologist other than the one who issued the original diagnosis. This is an opportunity for a second opinion on the diagnosis and provided the highest yield for detecting interpretive errors. Our study highlights how evaluation of studies used for prediction of response to treatment, rather than for diagnosis, can be an important mechanism to detect diagnostic errors intradepartmentally in a timely fashion.

183 Increased T-Lymphocytes, Both CD4 (+) and CD8 (+), Are Associated With Significantly Better Disease-Free and Overall Survival in Triple Negative Breast Cancer

Jabed Iqbal, Hirofumi Matsumoto, Aye Aye Thike, Puay Hoon Tan. Singapore General Hospital, Singapore, Singapore.

Background: Tumor infiltrating lymphocytes (TILs) signify immune response to tumor in a variety of cancers including breast cancer. However, earlier studies examining the clinical significance of TILs in breast cancers have generated conflicting results. There are very few studies that have addressed the relationship between TILs and clinical outcome in triple negative breast cancer (TNBC).
Design: Aim of this study is to evaluate the clinical significance of TILs, both CD4+ and CD8+, in TNBC. Immunohistochemical staining of CD4 and CD8 was performed on

a tissue micro array of 232 TNBC cases. TILs were counted separately as intratumoral when within cancer cell nest (iTILs) and as stromal when within cancer stroma (sTILs).
Results: High CD8+ iTILs and sTILs, and CD4+ iTILs correlated with histologic grade. According to Kaplan-Meier analysis, a significant better survival was observed in high CD8+ iTIL (DFS: *P*=0.004, OS: *P*=0.02) and both high CD4+ iTILs (DFS: *P*=0.025, OS: *P*=0.023) and sTILs (DFS: *P*=0.01, OS: *P*=0.002). Presence of high CD4/high CD8 TILs showed significantly longer survival compared with low CD4/low CD8 TILs (DFS in iTILs: *P*=0.023, OS in iTILs: *P*=0.027; OS in sTILs: *P*=0.021). In multivariate analysis, CD8+ iTILs (DFS: *P*=0.0095), CD4+ sTILs (DFS: *P*=0.0084; OS: *P*=0.0118), and high CD4/high CD8 iTILs (DFS: *P*=0.0121; OS: *P*=0.0329) and sTILs (DFS: *P*=0.0295) showed significantly better survival outcome.
Conclusions: These results suggest that presence of high levels of both CD8+ iTILs and CD4+ sTILs as well as high CD4+/high CD8+ iTILs and sTILs are independent prognostic factors in TNBC.

184 DNA Methylation in DCIS: Relation To Specimen Type, Histopathologic Features, and Development of Invasive Cancer

Erik Jenson, Kevin Johnson, Tracy Onega, Brock Chistensen, Jonathan Marotti. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Background: DNA methylation alterations have been shown to be early events in breast carcinogenesis, however, the relation of such epigenetic alterations to the histopathologic features of DCIS and development of invasive cancer remain unclear. Furthermore, the impact of a biopsy procedure on the epigenetic state of DCIS in future specimens and concordance between the two samples has not been fully evaluated.
Design: Whole-genome DNA methylation profiles of estrogen receptor positive DCIS (n=55) from 40 patients within the New Hampshire Mammography Network were measured using the Illumina HumanMethylation450 array. 13 patients subsequently developed invasive cancer with a median clinical follow-up of seven years for this cohort. Central pathology slide review was performed to record DCIS grade and architectural pattern, as well as presence or absence of prominent chronic inflammation, periductal fibrosis, calcifications, and necrosis. DNA was isolated from guided 2 mm cores of paraffin-embedded tissue blocks. Paired biopsy and surgical specimens were available for testing in 15 patients.
Results: Using linear regression models fit genome-wide, a CpG island located near the transcriptional start site of the *APC* gene, a known tumor suppressor gene, demonstrated significant (22 CpG loci at P<0.01) hypermethylation in high grade DCIS compared to low/intermediate grade DCIS. However, no significant enrichment was observed for any of the evaluated histopathologic features in patients that later developed invasive carcinoma compared to those who did not. Linear mixed effects models fit to each of 397,000 CpG sites did not identify significant methylation alterations between paired core biopsy and surgical specimens.
Conclusions: The identification of significant site-specific hypermethylation in high grade DCIS demonstrates that distinct methylation patterns exist among nuclear grades, which warrants further study. In matched samples, the biopsy procedure does not appear to influence patterns of methylation alterations in DCIS, an important finding for future methylation studies.

185 HER2 Results in Breast Carcinomas Following Updated ASCO/CAP Recommendation Guidelines

Ashley Jones-Ragle, Emily Reisenbichler. Vanderbilt University, Nashville, TN.

Background: Human epidermal growth factor receptor (HER)2 status in breast carcinoma is predictive of response to targeted chemotherapeutic agents. In 2007, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) established guidelines for uniformity in HER2 testing. The latest 2013 guidelines altered interpretation thresholds of HER2 results to increase testing sensitivity.
Design: Primary and metastatic breast carcinomas tested for HER2 by fluorescence in situ hybridization (FISH) between January 2013 and June 2014 were identified by a pathology report search. Primary tumor size, histologic type, grade, estrogen and progesterone receptor (ER and PR) status were recorded.
Results: FISH was performed on 638 cases, 335 and 303 of which were interpreted by 2007 and 2013 guidelines respectively (Figure 1). Numbers of not amplified cases decreased significantly under new guidelines while equivocal and amplified cases increased. The interpretation of 34 cases (11%) was changed by new guidelines (Table 1). Most cases with upgraded interpretations using new guidelines were high (57%) or intermediate (38%) grade carcinomas. Cases downgraded to not amplified were more frequently low to intermediate grade, ER/PR positive (75%) carcinomas.

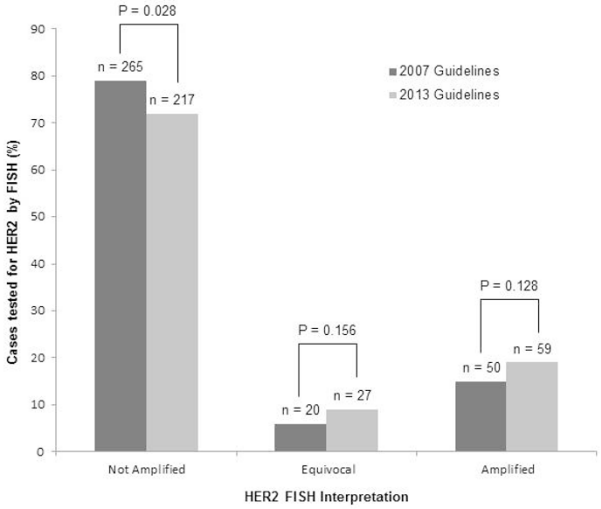


Figure 1: Comparison of the distribution of FISH results interpreted under 2007 and 2013 guidelines.

Table 1: Cases With Changed HER2 Interpretations Under New Guidelines

2007 Guideline Interpretation	2013 Guideline Interpretation	Tumor Tested	Primary Tumor Combined Histologic Grade	Primary Tumor ER/PR Status
Not Amplified	Equivocal	15 primary tumors 0 metastatic tumors	1 low 4 intermediate 6 high	13 positive 2 negative
Equivocal	Amplified	10 primary tumors 5 metastatic tumors	0 low 4 intermediate 6 high	5 positive 5 negative
Equivocal	Not Amplified	4 primary tumors 0 metastatic tumors	2 low 1 intermediate 1 high	3 positive 1 negative

Conclusions: Although the overall number of equivalent and amplified cases were increased by the higher sensitivity of the new guidelines, this change was not statistically significant. Cases changed by the new guidelines and now considered amplified by new thresholds are more frequently high grade while those now interpreted as not amplified are more frequently low grade.

186 Comparison of Estrogen, Progesterone Receptors, Ki-67 Index and Her-2/neu Status in Breast Cancer Before and After Neoadjuvant Chemotherapy

Shivani Kandukuri, Sophia Apple. University of California, Los Angeles, CA.

Background: Neoadjuvant chemotherapy (NAC) in breast cancer to decrease tumor volume for appropriate resection. Prior to NAC, Estrogen (ER) receptor, Progesterone (PR) receptor and Human Epidermal growth factor Receptor 2 (Her-2/neu) status by immunohistochemistry (IHC) and FISH on the core needle biopsy (CNB) are necessary to access sensitivity and for targeted therapy. Biomarkers however, may change after NAC which will alter further adjuvant systemic treatment management. Our study aims to identify the changes in the hormone receptors including ER and PR, Her-2/neu by IHC and FISH and Ki-67.
Design: A power path database search of all breast cancer cases between the years 2004 to 2014 was performed for cases with residual tumor after NAC. A total of 93 cases were identified where biomarker studies were performed on both pre- and post NAC specimens. ER and PR were considered positive when the tumor cells were staining ≥1 % and negative in <1%. The Ki-67 index was categorized into a three-grade system: < 10% (low), > 10-<20% (intermediate), and > 20% (high). Her-2/neu was considered positive when IHC 3+ staining was seen in >10% of tumor cells, 2+ as equivocal and 0 and 1+ were considered negative. FISH amplification cutoff value of ≥2.0 was positive and <2.0 was negative for amplification.
Results: Changes in ER are noted in 12/93 (13%) and in PR 20/93 (22%). The Ki-67 index changes are noted higher to lower values in most of the cases (46%) and were due to decrease in mitotic figures after NAC. Her-2/neu by IHC changed in 18/80 (23%) and by FISH 5 (7%) most commonly from positive to negative.

Biomarker (n= cases with available data)	+ to -	- to +	Equivocal to -	Equivocal to +	- to equivocal	Total changes (N=cases with change)
ER (n=93)	8	4				13%(N= 12)
PR (n= 91)	16	4				22%(N= 23)
Ki-67 (n= 71)	32	1				46%(N=33)
Her-2 IHC(n=80)	3		11	1	3	23%(N= 18)
Her2 FISH (n= 67)	4	1				7%(N=5)

Conclusions: - While tumor heterogeneity cannot be entirely excluded as a cause for variation in biomarker expression, our study showed significant changes in ER, PR, Her-2/neu and Ki-67 after NAC.
- Repeating biomarker studies post NAC for appropriate adjuvant chemotherapy is recommended.

187 Strong Association of Loss of p27 Expression and c-Myc Overexpression With High Grade Triple Negative Breast Ductal Cancers in African American Women

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Background: p27 inhibits progression from G1 to S phase. c-Myc plays a key role in cell cycle regulation and apoptosis. The objective of our study was to correlate the expression of c-Myc and loss of p27 by immunohistochemistry (IHC) in the four major subtypes of breast cancer (BC) (Luminal A, Luminal B, HER2, and Triple Negative) in a population of 202 African-American (AA) women with other clinicopathological factors including grade, stage, disease-free, and overall survival.

Design: Tissue microarrays (TMAs) were constructed from FFPE tumor blocks from primary ductal breast carcinomas in 202 AA women. Two separate 1 mm cores represented each case. The polymer-HRP system was utilized for immunostaining. Five micrometer sections were stained with a mouse monoclonal antibody against p27Kip1 (SX53G8, Cell Marque, Rocklin, CA). Additional five micrometer sections were stained with a rabbit monoclonal antibody against c-Myc (EP121, Cell Marque, Rocklin, CA). The sections were evaluated for intensity of nuclear reactivity (1-3) and percentage of reactive cells; an H-score was derived from the product of these measurements. Cases were categorized as having decreased (score $\leq$50) or increased (score >50) nuclear expression. Bivariate analysis was done via c2 analysis and survivability data was calculated via the generation of Kaplan-Meier curves (SPSS v19). Statistical significance was assumed if $p < 0.05$.

Results: Loss of p27 expression and c-Myc overexpression showed statistical significance with with ER negative ($p<0.0001$), PR negative ($p<0.0001$), triple negative (TN) ($p<0.0001$), grade 3 ($p=0.038$), and overall survival ($p=0.047$).

Conclusions: In our study, a statistically significant association between p27 loss and c-Myc overexpression and TNBC with decreased overall survival was found. A recent study found that constitutive c-Myc expression is associated with inactivation of the axin 1 tumor suppressor gene. p27 inhibits CDK2/cyclin A/E. Loss of p27 expression and c-Myc expression detected by IHC strongly suggest that cell cycle dysregulation is critical in the pathogenesis of TNBC in AA women. Axin 1 and CDK inhibitors may represent possible therapeutic targets for TNBC.

188 Correlation of Manual and Image Anlysis Ki-67 Scores in Breast Cancer Tissue Microarrays and Comparison With PAM50 Data

Thaer Khoury, Gary Zirpoli, Stephanie Cohen, Joseph Geradts, Melissa Troester, Christine Ambrosone. Roswell Park, Buffalo, NY; University North Carolina, Charlotte, NC; Duke University, Durham, NC.

Background: Ki-67 has increasingly been used in breast cancer (BC). It has variable staining patterns making morphologic interpretation challenging. The purpose of this study was to evaluate various staining patterns, compare them to image analysis, and to correlate the scores with a group of BC that were subtyped based on the PAM50 assay.

Design: A total of 22 BCs were molecularly subtyped using PAM50: luminal A (LumA) (n=6), luminal B (LumB) (n=10), HER2 (n=4), basal-like (n=2) and normal-like (n=1). Four one-mm cores from each tumor were randomly assembled in two tissue microarray (TMA) blocks. Ki-67 staining (MIB1, DAKO) was performed on an automated Bond immunostainer (Leica). The slides were manually scored by one breast pathologist. Image analysis (Aperio XT) was performed for all cells first and then using the Genie algorithm that enriched for epithelial cells. Four types of staining patterns were recognized and manually scored: mitotic figure or diffuse chromatin (DC), nucleolar only (Nuc), both nucleolar and chromatin (NC) and weak. A maximum of 500 cells were counted from each core. We evaluated tumor heterogeneity in the luminal types (n=16). The tumor was considered heterogeneous if at not all cores were either below or above the 14% cutoff that was arbitrarily chosen to differentiate LumA from LumB tumors. Tumor infiltrating lymphocytes (TIL) were subjectively scored using a scale from 0 to 3.

Results: 76 of 88 (86.4%) cores had at least 500 cells. The cores that had <500 cells were divided among 6 cases. Summing the count of cells in 2 randomly selected cores, 4 cases had <500 cells. The order of the highest percentage of positive Ki-67 was basal-like>HER2>Luminal types. When cases with a TIL score of >1 were excluded, the order of tumor subtypes was not affected. Tumor heterogeneity was seen in 3 LumB and 2 LumA cases. Table1 presents Pearson correlation coefficient between manual and image analysis.

Conclusions: When evaluating Ki-67 expression in a TMA setting, a 1-mm core diameter includes at least 500 cells in the majority of the cases. However, more than one core from each case is recommended due to tumor heterogeneity and to maximize the number of cases that include at least 500 cells. Excluding certain staining patterns could improve the correlation between manual scoring and image analysis.

	Image analysis (all cells)	Image analysis (epithelial cells)
Manual (all cells)	86.4%	91.6%
Manual (exclude NC/Nuc/Weak)	88.9%	92.3%

189 The Volume of Breast Atypical Ductal Hyperplasia in MRI-Guided Core Needle Biopsy Predicts Upgrade in Subsequent Excision: A Study of 100 Cases From Four Academic Institutions

Thaer Khoury, Zaibo Li, Souzan Sanati, Beatriu Reig, Prasanna Kumar, Mohamed M Desouki, Xiwei Chen, Dan Wang, Song Liu, Rouzan Karabakhtsian. Roswell Park Cancer Institute, Buffalo, NY; Ohio State University, Columbus, OH; Washington University, St. Louis, MO; Montefiore Medical Center, New York, NY; Vanderbilt University, Nashville, TN.

Background: Multiple histologic and radiologic variables have been found to predict upgrade for mammographically detected atypical ductal hyperplasia (ADH). The purpose of the study is to identify variables that can predict upgrade for MRI-detected ADH.

Design: We reviewed 1655 MRI-guided core needle biopsies (CNB) from four different academic institutions between 2005 and 2013 yielding 100 (6%) cases with ADH. The pathologic features were assessed including number of ADH foci, size of largest focus, total number of cores, number of involved cores, size of the biopsy needle, and presence of lobular neoplasia (LN). MRI findings including mass-enhancement vs. non-mass-like-enhancement, and the reason for biopsy were also recorded. An upgrade was considered when the subsequent surgical excision specimen yielded invasive carcinoma (IC) and/or ductal carcinoma in situ (DCIS).

Results: The rate of ADH in MRI-guided CNB among all four institutions ranged from 3.3% to 7.1%, with an average of 6%. A total of 15 (15%) cases had upgrade to DCIS (n=12) and IC (n=3). When all cases were included only increased number of involved cores was statistically significant ($p=0.02$). When cases with associated LN were excluded (n=14), increased number of ADH foci and increased number of involved cores were statistically significant ($p=0.002$ and 0.009, respectively). Data from a single institution which contributed the highest number of cases (n=61) was analyzed separately. We found that increased number of foci, increased number of total cores and involved cores and larger ADH size predicted upgrade (table).

Conclusions: The incidence of ADH in MRI-guided CNB is not uncommon. The rate of upgrade of ADH to DCIS or IC is comparable to mammographically detected ADH we previously reported. Therefore, surgical excision is recommended. Similar to mammographically detected lesions, the volume of the ADH predicts the upgrade. This can be used to guide therapy (excision vs. observation) in some clinical situations.

Variables	Upgrade Yes (n=8)	Upgrade No (n=53)	P value
Number of ADH foci 1(1,3)	2(1,3)	1(1,3)	0.015
Number of cores (total) 8(5,16)	6.5(5,8)	8(5,16)	0.026
Number of cores (involved) 1(1,2)	2(1,2)	1(1,2)	0.003
Largest ADH size (mm) 2.5(0.7,5)	3.5(1.5,4.5)	2.5(0.7,5)	0.021

190 Tumor Infiltrating Lymphocytes and Necrosis Are Independent Factors in Predicting Oncotype DX Recurrence Score in Breast Cancer

Thaer Khoury, Xiao Hwang, Xiwei Chen, Dan Wang, Song Liu, Mateusz Opyrchal. Roswell Park, Buffalo, NY.

Background: Pathology generated equations using standard morphologic parameters have been introduced to predict Oncotype DX recurrence score (ORS) in breast cancer. Tumor infiltrating lymphocytes (TIL) is known to indicate good prognosis in breast cancer, making the tumor more sensitive for chemotherapy. The purpose of the study is to correlate TIL and other morphologic parameters with ORS.

Design: Slides from 416 consecutive breast cancers with available Oncotype DX were reviewed. The following features were graded: Nottingham grade, necrosis (graded from 0 to 3), tumor/stroma ratio, degree of tumor infiltration, degree of TIL (graded from 0 to 3), and percentage of ductal carcinoma in situ. The following data were extracted from the pathology report: tumor size, estrogen receptor (ER) and progesterone receptor (PR) Allred scores, HER2 status, and ORS. Equation was calculated using ER, PR, HER2 and Nottingham grade. Then another equation was calculated using other significant morphologic variables.

Results: Table 1 illustrates the correlation between each of the clinical variables and ORS. The concordance rate using our equation that included ER, PR, HER2, and Nottingham grade was 69.61% for overall and 98.62% after excluding the intermediate category. Although multiple morphologic variables were statistically significant, only necrosis and TIL improved the equation. When these two variables were added to the previous equation, these rates became 70.1% and 98.63%, respectively. All patients (n=221) who were categorized as having low ORS had low or intermediate risk using our formula. Three of 44 (6.8%) patients who were categorized as having high ORS had low risk using our formula. However, one of these patients was treated with chemotherapy and developed bone metastases in 28 months.

Conclusions: TIL and necrosis are independent factors in predicting ORS. Adding these variables could improve existing equations. More studies are needed to examine the role of TIL in the setting of ORS assay. Is it a contaminant with the extracted tumor cells that falsely increased ORS or a true factor indicating chemo-sensitivity.

Variable	r	r2	p value
ER Allred score	-0.46	0.21	
PR Allred score	-0.58	0.34	
HER2	0.16	0.03	
Nottingham grade	0.32	0.1	
Necrosis	0.36	0.13	
Tumor/stroma ratio	0.15	0.02	
Tumor retraction	0.08	0.01	0.1
Tumor infiltration	-0.1	0.01	0.05
Tumor Infiltrating Lymphocytes	0.22	0.05	
DCIS%	-0.09	0.01	0.08

191 Ki67/BCL2 Index Is a Prognostic Predictor in Early-Stage Breast Cancer

Dong-Hoon Kim, Kyueng-Whan Min, Sung-Im Do, Seoung Wan Chae, Jin Hee Sohn, Jung-Soo Pyo. Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea.

Background: Considering the heterogeneity of breast cancer, it is particularly important to identify potential prognostic indicators. Ki67 and B-cell lymphoma 2 (BCL2) proteins are known prognostic markers in several cancers. Ki67 is associated with cell proliferation, whereas BCL2 has dual antiproliferative and antiapoptotic roles. A combinatorial maker based on these opposite functions might provide improved prognostic information.

Design: This study assessed Ki67 and BCL2 expression using tissue microarray obtained from 203 patients with invasive ductal carcinoma of the breast. A Ki67/BCL2 index based on the relative expression of each protein was dichotomised into high or low risk using receiver operating characteristic (ROC) curves.

Results: Ki67/BCL2 index significantly correlated with clinicopathologic parameters of age, stage, size, histologic grade, lymphatic and vascular invasion, necrosis, extensive intraductal component, estrogen receptor, progesterone receptor, HER2, response to adjuvant therapy, and p53 expression (all $p < 0.05$). Univariate and multivariate analyzes revealed a significant relationship between overall survival and Ki67/BCL2 index in early-stage breast cancer (all $p < 0.05$)

Overall survival	Univariate significance*	Multivariate significance†	Hazard ratio	95% CI
Ki67/BCL2 index (low risk vs. high risk)		0.04	2.19	1.036-4.631
AJCC stage (I or II vs. III)		0.054	1.922	0.989-3.732
Histologic grade (1 or 2 vs. 3)		0.157	1.703	0.815-3.56
Lymphatic invasion (absence vs. presence)	0.001	0.375	1.473	0.626-3.464
Perineural invasion (absence vs. presence)			3.605	1.862-6.981

[table1] Overall survival analyses correlated with Ki67/BCL2 index

Conclusions: A combinatorial index using Ki67 and BCL2 could be a meaningful indicator for predicting aggressive tumour behaviour in breast cancer, especially in patients with early-stage disease.

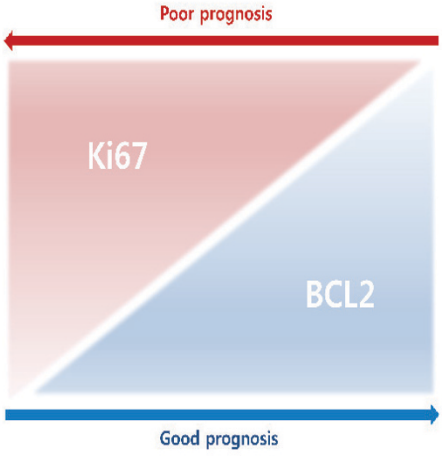

FIG. 1

192 Clinicopathological Significance of Dual Specificity Protein Phosphatase 4 (DUSP4) Expression in Invasive Ductal Carcinoma of the Breast

Hyunsung Kim, Se Min Jang, Jongmin Sim, Hyein Ahn, Kijong Yi, Yumin Chung, Hulin Han, Abdul Rehman, Min Sung Chung, Kiseok Jang, Seung Sam Paik. Hanyang University, Seoul, Republic of Korea.

Background: Dual specificity protein phosphatase 4 (DUSP4), known as mitogen-activated protein kinase phosphatase 2 (MKP2), is a member of the inducible nuclear MKP group. The role of DUSP4 in cancer development and progression appears to vary with the type of malignancy. The purpose of this study was to investigate DUSP4 expression in a series of invasive ductal carcinoma of the breast.

Design: We constructed tissue microarrays consisting of 16 cases of normal breast tissue, 14 cases of usual ductal hyperplasia, 47 cases of ductal carcinoma in situ, and 266 cases of invasive ductal carcinoma and investigated DUSP4 expression by immunohistochemistry.

Results: Cytoplasmic DUSP4 expression was observed. DUSP4 was more frequently expressed in malignant cases than in benign cases ($p = 0.024$). Mean DUSP4 expression score was significantly higher in malignant cases than in benign cases ($p = 0.019$). DUSP4 expression was significantly correlated with larger tumor size (more than 2 cm) ($p = 0.015$). There was no significant correlation between DUSP4 expression and overall survival or disease-free survival in all 266 patients. We evaluated the impact of DUSP4 expression on survival in 120 patients with T1 stage tumor. Interestingly, Kaplan-Meier survival curves revealed a significant effect of DUSP4 expression on both overall patient survival ($p = 0.034$, log-rank test) and disease-free survival ($p = 0.045$, log-rank test). In early T stage breast cancer, DUSP4 expression was a significant prognostic factor.

Conclusions: DUSP4 is frequently upregulated in breast cancer and may play a role in carcinogenesis and cancer progression and may be a marker of adverse prognosis, especially in patients with early T1 stage cancer.

193 Tyrosine Kinase Discoidin Domain Receptors DDR1 and DDR2 Are Coordinately Deregulated in Triple Negative Breast Cancer

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Background: The interaction of tumor cells with the collagenous extracellular matrix (ECM) is central to cancer progression. Discoidin Domain Receptors (DDRs) 1 and 2 are the only receptor tyrosine kinases that recognize collagen as ligands, and mediate collagen-initiated signaling. In cancer, DDRs regulate cell migration and invasion. Despite advances in the understanding of basic mechanisms of DDR1 and 2 function, there are limited data on the expression of DDR1 and DDR2 proteins in normal breast tissues and in different stages of breast cancer progression.

Design: We investigated the expression levels and patterns of DDRs in tissue samples of normal breast, ductal carcinoma in situ (DCIS), and invasive carcinomas from 218 patients. Clinical information and pathological features were recorded for all patients. The expression of DDR1 and DDR2 was assessed by immunohistochemistry using anti-DDR1 (Santa Cruz Biotechnology, Cat#SC-532) diluted 1:425 or mouse monoclonal anti-DDR2 (R & D Systems, Cat#MAB2538) diluted 1:75. Expression of DDR1 and DDR2 proteins was evaluated for: 1. Cell type and intracellular localization; 2. Intensity of staining (scores 1-4); and 3. Pattern of staining (focal vs. diffuse). The association between DDR expression and the clinicopathological characteristics of patients in our cohort was analyzed by using chi-square or Fisher's exact test. Survival was determined using Kaplan-Meier analyses.

Results: In normal epithelial cells and DCIS, DDR1 was expressed in the cytoplasm and cell membrane, while DDR2 was localized to cells at the epithelial-stromal interface. Of the 198 invasive carcinomas, DDR1 was low in 103 (52%), while DDR2 was high in 110 (55.6%). High DDR2 was associated with high tumor grade (p=0.002), and with the triple negative cancer subtype (TNBC) (p<0.0001), where it was accompanied by low DDR1 (p=0.003). The $DDR1^{low}/DDR2^{high}$ phenotype was significantly associated with worse overall survival (p=0.007).

Conclusions: We present the first comprehensive characterization of the concordant expression of DDR1 and DDR2 in breast tissue samples. We identified a previously unknow inverse association between low DDR1 and high DDR2 expression which is associated with survival in patients with breast cancer, and warrants further validation. Our study that may aid in defining the potential of DDRs as biomarkers and/or drug targets in specific breast cancer subtypes.

194 Updated HER2 In Situ Hybridization (ISH) Guidelines in Breast Cancer: Some Equivocal Findings

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Background: With the 2013 updated ASCO/CAP guidelines, dual-probe ISH assays saw a redefining of the equivocal category as an average HER2 count of 4-6 signals/cell with a HER2/CEP17 ratio < 2, a new positive category of ≥ 6 HER2 signals despite a ratio < 2 and a cutpoint for positivity of a ratio ≥2 (any HER2 count). We evaluated the impact of these changes on our laboratory practice and patient management.

Design: HER2 assay data were extracted from the electronic pathology reporting system for all breast cancers that underwent HER2 assessment by ISH since the 2013 update publication.

Results: During the study period, 306 tumours were tested by ISH [299 FISH; 4 bright-field dual ISH (DISH); 3 BOTH]. Of these, 65 (21.2%) were amplified, 209 (68.3%) were nonamplified and 32 (10.5%) were equivocal for HER2 gene amplification (Table 1). Of equivocal cases, 1 case of initial ISH testing had subsequent HER2 IHC which

was negative; 9 had a repeat ISH test: 2 cases had FISH and DISH performed on the same block and 8 had a second tumour block tested. Retesting of a second block showed HER2 amplification in 2 of 8 (25%) cases, a negative result in 2 (25%) cases, and an equivocal result in 4 (50%) cases. Retrospectively applying 2007 definitions resulted in a reassignment of category in 55 (18%) cases: 6 negative and 15 equivocal cases by 2007 guidelines were now considered positive, 9 cases equivocal by 2007 guidelines were now negative and 25 cases negative by 2007 guidelines fell into the 2013 equivocal category. There was no appreciable difference in equivocal cases between the 2007 and 2013 guidelines (31 vs 32); however, only 7 cases were equivocal using both definitions. Overall, 8 (2.6%) patients were now eligible for anti-HER2 therapy who would not have been previously.

Table 1. HER2 assessment by ISH using 2007 and 2013 ASCO/CAP guidelines^

		2007 Guidelines					
		Negative	Equivocal		Positive	Total	Eligible for anti-HER2 therapy:
			Ratio < 2	Ratio ≥ 2	Ratio ≥ 2.2		
2013 Guidelines	Negative*	200	9	-	-	209	NO (241)
	Equivocal*	25	7	-	-	32**	
	Positive* Ave HER2 ≥ 6 Ratio < 2	6	2	-	-	8	YES (65)
	Positive* Ratio ≥ 2	-	-	13	44	57	
	Total	231	18	13	44	306	
	Eligible for anti-HER2 therapy:	NO (249)		YES (57)			

^ IHC breakdown of cases sent for ISH: 228 equivocal (2+), 17 negative (0/1+), 4 positive (3+), 57 not performed
* On initial assessment
** On repeat testing 2 tumours were positive, 2 were negative and 4 remained equivocal

Conclusions: The HER2 guidelines update resulted in a change in HER2 status in 18% of tumours that underwent ISH testing (6.8% reclassified as HER2+) and affected eligibility for anti-HER2 therapy in 2.6% of patients. An increase in equivocal rates was not seen, although, there was little overlap in the cases deemed equivocal using different definitions. While the numbers are small, retesting a second tumour block may reassign 50% of equivocal cases into an amplified or nonamplified category.

195 Diagnostic Sensitivity and Utility of GATA3 in Breast Cancer (BC) After Hormonal (HT) and Chemotherapy (CT)

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Background: GATA3 is increasingly used for BC diagnosis. GATA3 is associated with estrogen receptor(ER) signaling but is also expressed in treatment(tx)-naive triple negative BC(TNBC) and is more sensitive than mammaglobin(MG) or GCDFP15(G15) in all BC subgroups. Neoadjuvant and adjuvant HT and CT may alter BC immunophenotype including ER and downstream genes, but the diagnostic sensitivity and utility of GATA3 with MG and G15 have not been evaluated in the post-tx setting in TNBC or other subgroups.

Design: 58 BC were immunostained for GATA3, MG and G15, including 8 pre/postCT HER2+ pairs, 12 pre/postCT TNBC pairs and 26 non-paired postCT TNBC, as well as 12 pre/postHT ER+HER2- pairs. Of 38 CT treated TNBC, 15 were biopsies or fine needle aspirates(FNA) of recurrences or metastases(RM). Any nuclear(GATA3) or cytoplasmic(MG, G15) staining was scored positive.

Results: GATA3 was positive in 87% of CT treated BC, compared to 56% MG and/or G15(p=.001). 81% CT treated MG-/G15- BC were GATA3+. GATA3 was positive in 84% CT treated TNBC, versus 49% MG and/or G15(p=.001). 80% CT treated MG-/G15- TNBC were GATA3+. Results were similar if only including RM. Two(20%) paired TNBC lost GATA3 after CT(p=.32). In CT treated HER2+BC, GATA3(100%) was more sensitive than G15(38%,p=.03) but not MG(75%,p=.47). After CT, GATA3, MG or G15 were positive in 93% BC, including 92% TNBC. 100% HT treated ER+HER2-BC were GATA3+ versus 75% MG+(p=.47) and 67% G15+(p=.48). No changes were seen with HT.

GATA3, MG and G15 in BC After CT or HT							
		GATA3	MG	p(GATA3 vMG)	G15	p(GATA3 vG15)	GATA3+, MG+ or G15+
postCT	Total %(n)	87(45)	44(47)	24(46) 93(45)			
	TNBC, all %(n)	84(37)	39(39)	21(38) 92(37)			
	TNBC, RM %(n)	92(13)	31(13)	.004	8(13)	92(13)	
	HER2+ %(n)	100(8)	75(8)	.47	38(8)	.03	100(8)
postHT	ER+ HER2- %(n)	100(12)	75(12)	.47	67(12)	.48	100(12)

GATA3, MG and G15 in CT treated BC Pairs						
	preCT			postCT (p, pre v post)		
	GATA3	MG	G15	GATA3	MG	G15
Total %(n=19)	95	35	15	84(.34)	58(.21)	32(.27)
TNBC %(n=11)	92	17	8	73(.32)	46(.19)	27(.32)
HER2+ %(n=8)	100	63	25	100(1.00)	75(1.00)	38(1.00)

Conclusions: GATA3 retains increased sensitivity over MG and/or G15 in CT treated TNBC, including biopsies and FNAs of RM. An immunopanel of MG, G15 and GATA3 shows optimal sensitivity for CT treated TNBC. HT does not alter GATA3 expression or sensitivity in ER+HER2-BC.

196 Immunophenotypic Switching of Biomarkers (IS) in Breast Cancer (BC) After Neoadjuvant Chemotherapy (NCT)

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Background: Molecular and immunophenotypic BC subgroups are clinically relevant. NCT may change ER, PR or HER2 expression with prognostic implications, suggesting a role for postNCT biomarker retesting. However, systematic evaluation of IS is lacking. We determined the frequency of IS in a large group of NCT treated BC and evaluated differences between subgroups.

Design: In 189 pre and postNCT samples, hormone receptor(HR;ER,PR) and HER2 protein expression(PE) were evaluated by immunohistochemistry(IHC) and HER2 amplification(AMP) by fluorescence in situ hybridization(FISH). ER,PR and HER2 scoring was based on ASCO/CAP guidelines. IS of PE was defined as change between + and - for HR and between -/equivocal(eq;0-2+) and 3+ for HER2. IS of HER2 AMP was defined as change between HER2-,eq and +.

Results: IS was seen in 29% of BC, including 16% with HER2 and/or ER IS. HR(22%) was more common than HER2(9%) IS (p<.001); PR(15%) was more common that ER(8%) IS (p=.05). Loss was more common than gain for PR(13 v 2%,p<.001) but not HER2(5 v 4%,p=.62) or ER(5 v 3%,p=.29). Of ER+BC, 7% became ER-; 9% ER-BC became ER+. The triple negative(TN) phenotype was lost in 12% TNBC, with 3% switching to HER2+ and 9% to ER+. Of HER2-BC, 6% became HER2+. Of HER2+BC, 27% showed decreased HER2 PE and 16% became HER2-. Of 10 HER2+BC that became HER2 IHC-, 20% switched to FISH- whereas 80% remained FISH+/eq. No differences in IS were identified between BC subgroups(p>.05).

Frequency of IS in NCT treated BC						
	All n=189	HER2+ n=64	HER2- n=125	ER+ n=137	ER- n=52	TN n=33
IS %	29	31	28	33	19	12
HER2 or ER IS %	16	20	14	15	19	12
HER2- to + %	4	-	6	4	2	3
HER2+ to - %	5	16	-	4	8	-
ER- to + %	3	3	2	-	9	9
ER+ to - %	5	2	7	7	-	-
PR- to + %	2	5	0	2	2	0
PR+ to - %	13	11	14	18	-	-

HER2+BC with HER2 IHC- After NCT				
	preNCT		postNCT	
	IHC	FISH	IHC	FISH
1	3+	+	1+	eq
2	3+	eq	1+	eq
3	3+	+	1+	+
4	2+	+	1+	+
5	2+	+	1+	+
6	2+	+	0	-
7	3+	NA	1+	+
8	2+	+	1+	+
9	2+	+	0	-
10	3+	NA	1+	+

Conclusions: IS is common in BC after NCT, including both gain and loss of HER2, ER and PR. BC subgroups show no significant differences in IS. HER2+ BC may selectively decrease HER2 PE compared to HER2 AMP, with implications for assay choice in the postNCT setting.

197 SATB2 Expression in Metaplastic Carcinoma of the Breast (MCB) With Osseous Differentiation (OD)

Gregor Krings, Yunn-Yi Chen. University of California, San Francisco, CA.

Background: SATB2 is a sensitive and specific marker for mesenchymal tumors with osteoblastic differentiation. MCB is a rare aggressive breast cancer (BC) subtype with poor chemotherapy response, which can display heterologous elements including OD. Diagnosis of MCB can be challenging in biopsies or fine needle aspirates (FNA) due to focality of keratin staining and focal or diagnostically subjective heterologous matrix production, including osteoid or bone (OB). SATB2 may be useful in this context but has not been evaluated.

Design: 30 MCB were immunostained with SATB2, including 8 with OD, 13 with chondroid differentiation, and 4 spindle cell carcinomas. 2 MCB with OD were metastases with OB in the primary but not metastatic site; 1 of these was FNA. MCB with OD were also immunostained with cytokeratin (panK) and GATA3. All staining was scored as focal (<10%), patchy (10-50%) or diffuse (>50%). Staining was scored separately in epithelioid and spindled cells and in areas with and without OB. Results were compared to tissue microarrays of 47 triple negative invasive ductal carcinomas (IDC).

Results: SATB2 was positive in all 8 (100%) MCB with OD (27% of all MCB), and negative in all 22 MCB without OD. In MCB with OD, SATB2 staining was diffuse in both epithelioid and spindled cells, and in areas with and without OB (n=8), including 2 metastases without OB. In contrast, panK and GATA3 each showed only focal staining in 3 (37.5%) MCB with OD (p=.026 each). None (0%) of 47 IDC showed SATB2 staining (p<.001 vs MCB with OD).

SATB2 Expression in MCB with OD					
	SATB2 cases +	panK cases +	p (panK v SATB2)	GATA3 cases +	p (GATA3 v SATB2)
Total (n=8)	8/8	3/8	.026	3/8	.026
Epithelioid or spindled cells in areas without OB (n=8)	8/8	3/8	.026	1/8	.001
Spindled cells, without OB (n=5)	5/5	1/5	.048	0/5	.008
Epithelioid cells, without OB (n=5)	5/5	2/5	.167	1/5	.048
E or S cells in areas with OB (n=6)	6/6	3/6	.182	2/6	.061

Conclusions: SATB2 is a highly sensitive marker for MCB with OD and is more sensitive than keratin or GATA3 in this context. Among MBC, SATB2 is sensitive and specific for OD, but expression is not limited to areas of OB, being diffusely positive in epithelioid and spindled cells without histologic features of OD, including metastases. The findings may be especially useful for diagnosis in scant specimens lacking definitive OB or other features of OD.

198 Whole-Genome Profiling Helps To Prognostically Classify Phyllodes Tumors of the Breast

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Background: The aim of this study was to analyse a series of borderline and malignant phyllodes tumors of the breast (PTs) by whole-genome profiling to identify genomic markers that could help to recognize potentially malignant tumors within borderline tumors.

Design: DNAs from frozen samples of 58 PTs, 28 borderline and 30 malignant (2012 WHO classification), were analysed on the Human CNV370 BeadChip microarray (Illumina Inc.), containing 370 000 SNP markers.

Results: Thirteen PTs (22%), 8 borderline and 5 malignant, showed no chromosome copy number variations (CNV). Twenty PTs (34%), 8/30 borderline (27%) and 12/28 malignant (43%), showed 5 or more chromosomal imbalances.

The large-scale CNV frequently associated with malignant tumors were +7p (9/28), -10p (8/28), -3q (6/28), +8q (6/28), -10q (6/28), and with borderline tumors was -6q (8/30). Losses in 9p21.3, encompassing *CDKN2A/B* gene, were present in 3 malignant tumors only, whereas deletions of 13q, with a minimal region in 13q14.2 encompassing the *RB1* gene, were found in 9/30 borderline and 7/28 malignant tumors.

High-level amplifications were seen in 8 tumours (7 malignant, 1 borderline): in 7p in 3 tumors (including *EGFR* in two), 7q31.2 in one tumor (including *TFEC* and *MET*), 8q24.21 in one tumor (including *MYC*), 8q23.3 (including *CSMD3*), in one tumor.

None of the 13 PTs with no CNV presented recurrence, metastasis nor death. The PTs group with CNV presented recurrence in 8/45 tumors (18%) or died of the disease in 2/45 (4%).

Conclusions: Whole-genome profiling by SNP-arrays in PTs leads to identify a high number of CNV, gains of 7p and 8q, losses of 3q and 10, losses in 9p21.3 (*CDKN2A/B*), and the presence of amplifications, especially involving *EGFR*, as markers of potentially malignant tumors.

199 Study of ESR1 Expression and Estrogen Receptor Signaling Pathway in Breast Cancer Patients

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Background: Per 2010 ASCO clinical practice guidelines committee and CAP council on scientific affairs, estrogen receptor (ER) or progesterone receptor (PR) with >1% positivity by immunohistochemistry (IHC) should be considered positive, while the cut off value was 10% before. Research revealed that there was no significant difference in overall survival and recurrent-free survival among breast cancer patients with negative ER IHC, ER IHC 1-5% positivity or ER IHC 6-10% positivity. This showed that the relationship between the empirical threshold of ER IHC positivity and true underlying ER biological function was poorly elucidated. To date the RNA expression levels of estrogen receptor (ESR1) have not been completely defined in breast cancer patients. Since correct classification of ER status has clinical implications, we try to investigate the relationship between ER IHC levels and ESR1 expression in a 84-gene based ER signaling pathway to try to find a better way to classify ER status especially breast cancer with low ER IHC.

Design: We compared the ESR1 expression and ER protein levels in 19 triple negative breast cancer patients, 14 patients with ER IHC>50% positivity and 8 patients with ER IHC1%-10% positivity with up to 70 months' follow-up using formalin-fixed, paraffin embedded, blinded tumor samples. They were evaluated for gene expression in ER signaling pathway using RT² Profiler™ PCR Array expression kits containing 84 genes. Immunohistochemistry was used to evaluate ER protein status.

Results: Among 19 ER negative patients, 17 patients had down-regulated ESR1 (17/19=89%). Among 14 cases with ER IHC>50% positivity, 12 patients showed up-regulated ESR1 (12/14=86%). All eight patients with ER IHC 1-10% positivity had down-regulated ESR. The rate of ESR1 up-regulation in breast cancer with ER IHC 1-10% positivity is significant lower than that with ER IHC >50% positivity ($p<0.01$). All 12 breast cancer patients with ER IHC positive/up-regulated ESR1 survived during follow-up, while 3 of 10 patients with ER IHC positive/down-regulated ESR1 expired during follow-up with one patient died from non-breast cancer related reason. Two genes, CTSD and EFNA5, in ER signaling pathway were closely related to the ESR1 up-regulation ($p<0.05$).

Conclusions: ESR1 expression analysis may help to confirm the actual hormonal status in breast cancer cases with 1% to 10% ER IHC positivity. Accurate classification is essential to appropriate management and prognostication of breast cancer patients.

200 Digital Quantification of Estrogen Receptor Expression in Normal Breast in Post-Menopausal Women With Breast Cancer and Association With Tumor Subtypes

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Background: Estrogen Receptor (ER) expression in normal breast epithelium (NBR) is higher in women with a history of breast cancer (BC) compared to controls. The aim of this study is to quantify ER expression in NBR away from tumor in women with BC and to correlate it with BC subtypes.

Design: 204 BC patients were identified for whom NBR away from tumor was available. Tissue microarrays were constructed, stained with ER and scanned using Aperio XT Scan Scope. Normal terminal duct lobular epithelium was manually circled on scanned images and annotations were recorded in separate digital layers. ER was quantitated in marked areas of the electronic image using an optimized scoring nuclear IHC algorithm (Aperio technologies, Inc.). Clinical information and tumor characteristics (menopausal status, ER, HER2 expression, grade, size, number of positive nodes, stage) were recorded.

Results: The mean ER positivity in NBR was 16 ± 12.4 % (range: 0-5-5.7%) for all patients with BC, 20.8±13.9% for n=74 postmenopausal (PostM) and 13.4 ±10.9% for n=130 peri+premenopausal patients. ER positivity in NBR from BC patients did not vary by tumor size, positive lymph nodes status, tumor grade, or stage in PostM, and nor in peri+premenopausal women. Older age at diagnosis was significantly associated ($p<0.0001$) with ER in NBR. In PostM women ER expression in NBR was significantly higher in patients with ER negative or triple negative tumors.

		Postmenopausal (N=74)		
		N	Mean % (SD)	P Value
Tumor ER Status				
	Negative	14	27.4 (13.9)	
	Positive	60	19.3 (13.6)	
Tumor HER2 Status				0.34
	Negative	57	21.8 (14.9)	
	Positive	8	17.7 (5.7)	
Tumor Triple Negative				0.04
	No	53	19.7 (13.6)	
	Yes	12	28.5 (14.7)	

p-valuesare adjusted for age at diagnosis. HER2 status was not known in some cases and was excluded from statistical calculations.

Conclusions: This study, based on quantitation from digital images, confirms ER expression in NBR increases with age and menopausal status in women with BC. We report, for the first time, a significant association between ER expression in NBR with ER negative and triple negative cancers in PostM women.

201 Equivocating on Equivocals – HER2 Status Changes Following Implementation of the 2013 ASCO/CAP Guidelines: A National Reference Laboratory Experience

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Background: In 2013, ASCO/CAP published new guidelines for HER2 testing. The new guidelines have defined HER2 by immunohistochemistry (IHC) with >10% (previously >30%) strong circumferential membrane staining (CMS) as positive (3+). Equivocal (2+) cases are those with incomplete and/or weak/moderate CMS in >10% of cells or complete, intense, CMS in ≤ 10% (previously ≤ 30%) with reflex to in situ hybridization. We retrospectively reviewed cases submitted to our lab for HER2 testing to assess the effect of the 2013 guidelines on IHC 2+ cases, specifically cases with 10-30% CMS, reflexed to fluorescence in situ hybridization (FISH).

Design: Our lab offers HER2 IHC by Hercept (Dako) or 4B5 (Ventana) antibodies, and dual probe FISH (Abbot Molecular). HER2 IHC & FISH tests performed 1/2010-8/2013, originally scored with 2007 guidelines, were included in the study. IHC was manually read following 2007 guidelines. Intensity (weak, moderate, strong) and the % CMS was also recorded. FISH was manually scored and originally reported per 2007 guidelines. As part of the study, amplification (Amp) rates for IHC 2+ cases were re-evaluated using new guidelines by re-assigning cases with HER2/CEP17 ratio of ³2.0 or a HER2 average copy number ≥6/cell as Amp.

Results: During the study period, 4,145 HER2 IHC tests were performed and reported following 2007 guidelines. 1,088 (26.2%) of these were 2+ by IHC. Reflex FISH was requested on 731/1,088 (67.2%) of the 2+ IHC cases. Using the 2007 guidelines, 48 /707 (6.8%) of the 2+ cases were Amp, 23 (3.3%) remained equivocal, and 635 (89.8%) were not amplified (NA). An interpretable FISH result was not available in 24/731 (3.3%) of cases. 507(71.7%) cases had 10-30% CMS (8 strong; 136 moderate; 363 weak). HER2 Amp rates in these cases using the 2013 guidelines are shown in Table.

IHC2+		FISHAmplified (2013 Guidelines) (%)	
2+ Cases (All)		58/707 (8.2%)	
2+ Cases, with 10-30% Circumferential Membrane Staining:		38/507 (7.5%)	
	Strong		1/8 (12.5%)
	Moderate		17/136 (12.5%)
	Weak		20/363 (5.5%)

Conclusions: Amp rates for 2+ IHC cases with 10-30% strong and moderate CMS are similar although only the latter group is recommended to have FISH following new guidelines. FISH confirmation of IHC 3+ cases with low % CMS may be justified considering subjectivity involved in evaluating staining to avoid false positives potentially resulting in unnecessary treatment.

202 Pathologic Findings of Follow-Up Surgical Excision for Radial Scar on Breast Core Biopsy

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Background: Radial scar (RS) has been found to be associated with both benign breast lesions and malignant/atypical lesions. The data regarding RS as a risk factor for developing breast cancer are conflicting and it is controversy if an excision will be warranted for patients with radial scar identified on core needle biopsy (CNB). In this study, we investigated the follow-up excision results for patients with radial scar on CNB, but no prior or current high-risk factors.

Design: 403 cases of radial scar without any other concurrent or prior histologic abnormalities were included in this study. Follow-up excision (FUE) was performed in 220 (54.6%) cases. Demographic features, radiologic findings and pathologic findings were recorded. The majority of patients (91.5%, 369/403) were rendered a BI-RADS score of 4 and there was no significant difference in radiologic findings including the BI-RADS score between cases with FUE and cases without FUE.

Results: Out of 220 cases with radial scar on CNB, only one invasive carcinoma (IC) (0.5%) and one ductal carcinoma in-situ(DICS) (0.5%) were found on FUE. 44 cases (20.0%) were found to have atypical ductal hyperplasia (ADH) and 13 cases (5.9%) were found to have lobular neoplasm (LN) on FUE. We found the upgrade rate is associated with increased age, but not any other variables (clinical presentation, radiologic findings, etc).

lesions	Case #	%
Invasive cancer	1	0.5
DCIS	1	0.5
ADH/ADH+LN	44	20.0
LN	13	5.9
FEA	5	2.3
total upgraded cases	64	29.1
Not-upgraded cases	156	70.9
Total	220	100

Conclusions: This is one of the largest studies to evaluate excisional findings on patients with radial scar on CNB. Although the high-risk lesion (HRL) (ADH, LN) upgrade rate of RS following excision is about 26%, the carcinoma (DCIS, IC) incidence is extremely low (1%) in this study. Our data suggest RS is frequently associated with high-risk lesions (ADH, LN), but not carcinoma on CNB. Excision might not be necessary for those who have only RS on CNB and no other known risk factors, although clinical and radiological follow up might be warranted.

203 Genomic Profiling of Radial Scar

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Background: A radial scar in the breast is characterized by a stellate nidus with dense collagen associated with entrapped ducts and lobules. Although the lesion itself exhibits benign cytohistology, previous studies have indicated that the presence of radial scar is associated with concurrent high-risk lesions, such as atypical ductal hyperplasia, carcinoma in situ, or invasive cancer. While the radial scar has been described in the literature for decades, its pathogenesis and the underlying molecular mechanisms that links it to high-risk lesions remain largely unknown. Our lab has previously examined the genomic profiles and transcriptome of other early breast neoplastic lesions. In the current study, we performed transcriptome and exome analyses to understand the molecular mechanisms linking radial scars to high-risk lesions.

Design: RNA-sequencing and exome-sequencing were performed on archival FFPE blocks from two pilot cases with radial scar, three hyperplasia lesions, five DCIS, one invasive breast cancer, with 3 paired normal breast tissue. Somatic single-nucleotide polymorphisms and insertion-deletion gene mutations in the radial scar samples were analyzed and annotated by Samtools, VarScans, MuTect, and ANNOVAR. The results are compared with their mRNA expression profiles.

Results: Exome-sequencing of radial scars reveals multiple recurring, deleterious somatic mutations that are with high allele frequency and are not present in the paired normal breast tissue. Some of these targets are overlapped with the somatic mutations identified in paired DCIS sample, indicating the clonality. In addition, compared with data from The Cancer Genome Atlas (TCGA) of invasive breast carcinoma, the radial scars share known common mutations occurring in invasive breast cancer. The mRNA expression profiles of the candidate genes are generated, and the unsupervised hieratical clustering analysis shows clustering of radial scar samples.

Conclusions: Our study is the first to profile the somatic mutations and mRNA expression profiles of radial scars. We identified recurring somatic mutations, possibly representing the driver mutations in pathogenesis. These findings, together with the results of other high-risk breast lesions, will not only expand our knowledge in disease progression, but also provide novel diagnostic markers for identification of high-risk breast lesion.

204 Improved Detection for Breast Myoepithelial Cells By A New Clone of Smooth Muscle Myosin Heavy Chain Antibody

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Background: Immunohistochemistry (IHC) of myoepithelial cell (MEC) markers is routinely used in breast pathology for detecting the presence or absence of an invasive carcinoma. Smooth muscle myosin heavy chain (SMMHC) and p63 are among the most commonly used MEC markers and often used together as a panel. However, reduced SMMHC expression in MECs bordering the tumor cell nests in the ductal carcinoma in situ (DCIS) has been observed in several studies where clone SMMS-1, was applied. Our preliminary study showed that a rabbit monoclonal antibody against SMMHC, clone EPR5336(B), was highly sensitive and specific in in detecting MECs in breast lesions compared to clone SMMS-1.

Design: Clones EPR5336(B) and SMMS-1 were first optimized for IHC on FFPE human breast tissue sections; then they were compared on individual and microarray breast tissues. The microarrays consisted of normal mammary glands, benign lesions, carcinoma in situ and invasive carcinomas. Further analysis was done to compare clone EPR5336(B) with clone SMMS-1 on breast carcinoma in situ cases, with p63 antibody clone 4A4 as reference marker for MECs, on 16 cases of DCIS and 2 cases of LCIS.

Results: Clone EPR5336(B) produced more intensive staining than clone SMMS-1 in all breast tissues tested. While both clones stained almost all MECs in normal breast tissues and hyperplasias, clone EPR5336(B) produced much stronger staining intensity. In MECs bordering carcinoma in situ (CIS), SMMS-1 staining became very weak or even negative while the staining by clone EPR5336(B) was always a notch stronger. In 18 breast CIS cases that were stained by the two SMMHC clones and p63, clone EPR5336(B) demonstrated outstanding capability of highlighting MECs around the lesions. Clone EPR5336(B) stained MECs with complete circumference bordering CIS in 11/18 cases, with partial circumference in 6 cases. In contrast, clone SMMS-1only demonstrated complete circumference staining of MECs in 2 cases and partial circumference in 10 cases. There was only one case of CIS where MECs were not stained with clone EPR5336(B) while clone SMMS-1 failed to detect MECs in 6 cases. Although p63 was able to stain MECs around lesion of CIS in all cases, only 7 achieved staining of complete circumference.

Conclusions: The study demonstrates that clone EPR5336(B) of SMMHC antibody is more sensitive than clone SMMS-1 in detecting breast MECs.

205 The New Equivocal: Changes To HER2 FISH Results When Applying the 2013 ASCO/CAP Guidelines

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Background: Human epidermal growth factor receptor 2 (HER2, *ERBB2*) testing is an important prognostic and predictive marker in breast cancer management, especially in selecting HER2 targeted treatment. The determination of HER2 status is addressed in ASCO/CAP guidelines, recently revised in 2013, replacing the initial 2007 version. The 2013 guidelines significantly change in-situ hybridization (ISH) interpretation, specifically returning to the prior threshold of HER2/CEP17 ratio ³2.0 for positive, and eliminating 1.8-2.2 as the equivocal range. In addition, the HER2 signal/nucleus ratio is taken into account, with ≥6.0 considered positive and 4.0-6.0 considered equivocal even in cases with a HER2/CEP17 ratio <2.0.

Design: With IRB approval, we retrospectively reviewed our HER2 FISH results from 2006-2012, and classified them according to both the 2007 and 2013 guidelines as negative, positive or equivocal. During this time period, we performed FISH on cases with 1+ or 2+ immunohistochemistry results.

Results: Of 719 HER2 FISH results, 55 (7.6%) changed when re-assessed by the 2013 guidelines (Table 1). There was a major re-shuffling of the equivocal category. 19 of 25 results in the 2007 equivocal category, defined by a HER2/CEP17 ratio between 1.8 and 2.2, were re-assigned as either positive (13) or negative (6). Only six 2007

equivocal results remained equivocal when 2013 guidelines were applied. Since the update designates specimens with HER2 signal/nucleus ratio of 4.0-6.0 as equivocal, 35 previously negative cases became equivocal in the 2013 scheme, 12 of these with 1+ immunohistochemistry. One negative patient was reclassified as HER2 positive with low HER2/CEP17 ratio and HER2/nucleus=6.

2007 to 2013 Result Changes per ASCO/CAP Guidelines	Number	%
Remained negative	586	81.5%
Negative to equivocal	35	4.9%
Negative to positive	1	0.1%
Equivocal to negative	6	0.8%
Remained equivocal	6	0.8%
Equivocal to positive	13	1.8%
Remained positive	72	10.0%

Conclusions: The revised 2013 ASCO/CAP guidelines increased the number of HER2 FISH positive and equivocal results, as well as the number of patients that would have been considered for anti-HER2 therapy. The equivocal group is substantially different than under the 2007 guidelines, posing a dilemma for clinical management. These changes highlight the complexity added by considering both HER2/CEP17 and HER2/nucleus in interpretation of HER2 FISH.

206 Clinicopathological Features of Breast Carcinoma (BC) With PIK3CA Mutations

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Background: *PIK3CA* mutations are relatively common genetic aberrations in BC. We evaluated the clinicopathologic features of BC in a large cohort of patients with *PIK3CA* mutations.

Design: Tumors from patients with locally advanced or metastatic breast carcinoma were tested for *PIK3CA* mutations using the Sequenom or MSK-IMPACT platforms for a clinical study at our institution. Clinicopathologic features reviewed included patient age at diagnosis, primary tumor histologic subtype, grade, size, lymphovascular invasion (LVI), receptor status, nodal status, and time interval to distant metastasis.

Results: A total of 513 tumors were tested between 02/13 and 03/14. Receptor status was known for 422 BC: 297 (70%) were ER/PR+, HER2-; 55 (13%) ER/PR+, HER2+, 11 (3%) ER/PR+, HER2 equivocal (by IHC and FISH); 33 (8%) ER/PR-, HER2+; 26 (6%) ER/PR/HER2-. A total of 78 cases harbored *PIK3CA* mutations, including H1047R (38), E545K (16), E542K (12), H1047L (4), N345K (4), E545A (2), C420R (1) and H1047Y (1). Among cases with *PIK3CA* mutation, the primary BC was ductal (61), lobular (11), or mixed ductal and lobular (6). Tumor size ranged from 0.25-8.5 cm (average 2.9). Tumor grade was known for 41 BC, including 1 (2%) grade 1, 20 (49%) grade 2, 20 (49%) grade 3. LVI was present in 32/78 (41%). Lymph node status at diagnosis was known in 51 patients; 37 (73%) were node positive. We found no unique morphologic feature(s) associated with *PIK3CA* mutation. The time interval to metastasis ranged from 0-226 months (average 59 months). Receptor status was known in 68 BC with *PIK3CA* mutation: 51 (75%) were ER/PR+, HER2-; 5 (7%) ER/PR+, HER2+; 3 (4%) ER/PR+, HER2 equivocal (by IHC and FISH); 6 (9%) ER/PR-, HER2+; 3 (4%) ER/PR/HER2-. The distribution of specific mutations according to the receptor status is summarized in Table 1. One of the hotspot mutations E545K was detected only in ER/PR+, HER2- tumors in this cohort. Among BC without *PIK3CA* mutation, 13 cases harbored AKTE17 mutation.

Receptor Status	H1047R	E545K	E542K	Other	Total
ER/PR+, HER2-	23	13	5	10	51
ER/PR+, HER2+	5	0	0	0	5
ER/PR+, HER2 equivocal (by IHC and FISH)	2	0	0	1	3
ER/PR-, HER2+	4	0	2	0	6
ER/PR/HER2-	1	0	1	1	3
Total	35	13	8	12	68

Conclusions: *PIK3CA* mutation did not correlate with any specific morphologic features in the primary tumor. Response to PI3K inhibitors in this cohort of patients is under investigation.

207 Folate Receptor Alpha (FRA) Expression in Pregnancy Associated Breast Cancer (PABC)

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Background: Folate is required for cell metabolism, DNA synthesis and repair. The folate receptor regulates cellular folate homeostasis and cellular proliferation. Folate receptor alpha (FRA) is expressed in breast cancer; its expression is often associated with triple-negative (TN) phenotype and worse prognosis. Pregnancy associated breast cancers (PABC), which are diagnosed during or after a pregnancy, are also more likely to be TN cancers with a poor prognosis. In this study we evaluated FRA expression in PABC and correlated its expression with clinicopathologic features in this aggressive breast cancer subtype.

Design: 23 patients diagnosed with PABC within 2 years of pregnancy (mean age 35.8, range 26-48). Age-matched nulliparous women with breast cancer served as controls (mean age 37.5, range 29-51). Pathologic tumor features including histologic type, tumor grade, tumor markers (ER, PR, HER2) and lymph node (LN) status were assessed. Extent of FRA expression by immunohistochemistry (0=<1%, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%), staining intensity (0=absent, 1+=weak, 2+=moderate, 3+=strong) and product score (extent x intensity) were calculated. Staining pattern was noted as luminal, cytoplasmic, membranous or perinuclear.

Results: Overall, PABC cases were more likely than controls to have greater extent of FRA staining (3 staining: 26% vs 7%), to have strong staining (3+: 35% vs 20%) and to be associated with a TN phenotype (33% vs 7%). All (7/7, 100%) of the TN PABC cases showed strong FRA staining. Of interest, 30% of the PABC cases showed a membranous staining pattern, while none (0%) of the control cases did so. Furthermore, membranous staining PABC cases had a higher product score than non-membranous staining PABC cases (mean 7.57 vs 2.25, $p = 0.0013$). Finally a higher % of the membranous staining PABC cases were grade 3 tumors, had TN phenotypes and had positive LN compared to non-membranous staining PABC cases (85% vs 63%, 43% vs 25% and 86% vs 44% respectively).

Conclusions: 1. PABC are more likely to express FRA. **2.** PABC with TN phenotype show stronger intensity and greater extent of FRA. **3.** FRA staining pattern in PABC is more likely to be membranous. **4.** Membranous staining PABC are more likely to be higher grade tumors with TN phenotype and have positive LNs. Our findings of FRA expression in a membranous pattern in PABC adds to the understanding of the molecular pathways that operate in different breast cancer subtypes and may to lead to therapeutic strategies targeting the folate receptor expression in these aggressive tumors.

208 Adenoid Cystic Carcinoma and Basaloid Salivary-Like Tumors of the Breast. A Clinicopathologic Study

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Background: Adenoid cystic carcinomas (ACC) of the breast are rare tumors of excellent prognosis, with characteristic histology, and molecular features consisting in a recurrent chromosomal translocation t(6;9)(q22-23;p23-24), responsible for the fusion of MYB with NFIB, and overexpression of MYB. Basaloid salivary gland-like tumors, include basal adenomas (cylindromas), and basaloid carcinomas, which are possibly related to ACC. The aims of this study were to define the clinicopathologic features of these tumors and the diagnostic utility of MYB IHC.

Design: Twenty-five breast tumors, previously diagnosed as ACC or basaloid tumors, were reclassified histologically, and graded according to the degree of cytologic atypia, mitotic activity and growth pattern. Clinical data obtained included age, tumor size, lymph node status, and follow-up. IHC included stains for estrogen/progesterone receptors, HER2, p63, c-kit and MYB.

Results: Thirteen cases were classified as ACC, two as basal cell adenomas, and ten as basaloid carcinomas. Median age was 59.2 years (range. 34-78 years), median tumor size was 24.9 mm (range, 12-45 mm). All cases were triple negative; MYB IHC was positive in 13 (100%) ACC, two basal cell adenomas were positive but in scattered cells, and six (60%) basaloid carcinomas were positive. p63 was positive in all ACC and basal cell adenomas, and in four basaloid carcinomas. c-kit was positive in all the tumors. Lymph node metastases were present in one ACC (pN1a), and one basaloid carcinoma (pN1mi), two basaloid carcinomas were pN0 i+. One ACC developed brain metastases and died. One patient with basaloid carcinoma is alive with pleuropulmonary metastases.The remaining cases are alive without evidence of disease.

Conclusions: Tumors with basaloid features in the breast, including ACC, basal cell adenomas, and basaloid carcinomas, are within the same histological spectrum, and are probably genetically related. MYB stain was positive in a high percentage of cases. The prognosis of these tumors is good, with little tendency to lymph node metastases, but occasional cases may develop distant metastases.

209 Cellular Angiolipoma of the Breast: Series of 4 Cases, Morphological Characteristics, Immunoprofile and Review of Literature

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Background: Cellular angiolipoma is a rare entity in the breast that may present with a mammographic abnormality or palpable mass. To date, there are only sixteen cases reported in the literature with the largest study including 14 cases. Per the reports lesions were either in the breast parenchyma or subcutis of the breast.

Design: We searched the pathology database for all examples of angiolipoma of the breast, and reviewed the clinical presentation, imaging studies and follow-up data of those classified as cellular angiolipoma.

Results: A total of 116 cases of angiolipoma of the breast were retrieved from our surgical pathology archives for the period from1990 to 2014. Of these, four cases were classified as cellular angiolipoma (3.4%), all of which were from females. Comparatively, for the same time period, there were 1752 cases of extramammary angiolipoma, of which seven were classified as cellular angiolipoma (0.39%, P<0.0001). All seven extramammary cases were from the arm or chest. The demographic, clinical, and follow-up data are summarized in Table. The morphology in all of our four cases was typical with cellular spindle cell areas and vascular proliferation in a background of mature adipose tissue with characteristic intravascular fibrin thrombi and low mitotic rate (< 1 per 10 HPFs) and absence of necrosis and nuclear pleomorphism. Immunohistochemically they are positive for CD34, but negative for desmin and HHV-8.

Conclusions: Cellular angiolipoma is rare, but in our experience is more common in the breast than at other sites. They may be encountered as needle biopsies, but close attention to the morphologic features and use of ancillary studies will assist in distinguishing these lesions from Kaposi's sarcoma or angiosarcoma.

	Case 1	Case 2	Case 3	Case 4
Age (years)	73	74	72	45
Presentation	Mammographic abnormality	Palpable mass	Mass on mammography	Ill-defined mass on radiology
Location	Left breast 6 o'clock	Left 10o'clock subcutis mass	Left breast 9 o'clock, middle depth	Left breast 3 o'clock
Diagnostic method	Excision	Excision	Stereotactic core biopsy	Ultrasound guided needle biopsy
Size	4 mm	1.1 cm	1.8 cm	9 mm
Follow-up time	6 months	58 months	48 months	6 months

210 Androgen Receptor Expression in Brain Metastases From Breast Cancer

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Background: Approximately 15% of all patients with metastatic breast cancers have symptomatic brain metastases (BM), with a median survival of less than one year. The androgen receptor (AR) is expressed in many primary and metastatic breast cancers, and there is data that anti-androgen therapy may show promise in treating patients with AR+ disease. However, AR expression in brain metastases from breast cancer has not been well characterized.

Design: Tissue microarrays from a well-annotated cohort of breast cancers metastatic to the brain were evaluated for AR expression by IHC (clone SP107, Ventana Benchmark Ultra). AR positivity was defined as >=10% staining in tumor nuclei. Differences in AR expression between the breast and metastatic tumor were assessed in the subset of patients with matched tissue. Gene expression profiling was performed using macro-dissected FFPE material on Affymetrix HTA 2.0 arrays, and PAM50 subtypes were derived. AR expression was then correlated with ER/HER2 status and PAM50 molecular subtype.

Results: Overall, 29 of 67 BM (43%) were AR+. AR positivity was seen at a higher rate in patients with ER+ and/or HER2+ BM as opposed to patients who were ER-/HER2- (see table 1).

AR Status	ER+/HER2-	ER+/HER2+	ER-/HER2+	ER-/HER2-
Positive	10 (59%)	6 (60%)	8 (53%)	5 (20%)
Negative	7 (41%)	4 (40%)	7 (47%)	20 (80%)

Table 1. Comparison of AR and ER/HER2 expression in brain metastases.

When AR status was segregated by PAM50 molecular subtype, AR positivity was seen more often in the luminal A (65%) and B subtypes (67%) than in the ERBB2+ (25%) and basal (5%) subtypes. Interestingly, of the 15 cases with matched primary and metastatic tissue, 11 cases (73%) showed the same AR result for both the primary and BM (6 +/+, 4 -/-), while 4 cases (27%) showed a change from AR+ in the primary cancer to AR- in the metastases.

Conclusions: These findings demonstrate that many brain metastases from breast cancer express AR. AR positivity is seen more often in patients with ER+ and luminal cancers as determined by routine IHC parameters and molecular profiling. When comparing AR+ matched primary breast cancers with corresponding brain metastases, loss of AR expression in the brain metastasis is identified in close to a third of patients. The results indicate that targeted anti-androgen therapy may be a reasonable option for patients with brain metastases from breast cancer and that AR testing should be performed on the metastatic tissue.

211 Clinical Utility of ER and CK5 Separately or as a Cocktail in Separating ADH and Low Grade DCIS From UDH

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Background: Previous studies have looked at the use of high molecular weight cytokeratins (HMCKs) such as CK5 to help distinguish between usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH) and low grade DCIS. Low grade atypical proliferative lesions generally have diffuse estrogen receptor (ER) expression. Few studies, however, have looked at the utility of combining a HMCK with ER to differentiate ADH or low grade DCIS from UDH. The goal of this study is to assess the utility of CK5 and ER each as a single stain or as a cocktail, in separating ADH and low grade DCIS from UDH. In addition, the utility of Bcl-2 and PR expression was assessed.

Design: Archived pathology files identified the following study cases: 13 ADH, 10 low grade DCIS and 22 UDH. Tissue blocks were stained for ER, CK5, PR and Bcl-2. Nuclear staining for ER and PR was scored as diffuse (≥80%), focal (<80%), and negative (0%). Cytoplasmic staining for CK5 and Blc-2 was scored as diffuse (≥60%), focal (<60%), and negative (0%). Differences in staining between the study groups and the sensitivities and specificities were calculated.

Results: ER, CK5, and PR expression patterns were significantly different in ADH and low grade DCIS versus UDH ($p<0.001$). For ER: 10/13 (77%) ADH and 10/10 (100%) DCIS cases showed diffuse staining (sensitivity 87%, specificity 100%); none of the 22 UDH cases showed diffuse staining with ER. The combination of ER and CK5 versus either alone increased the sensitivity of identifying low grade atypical proliferative lesions to 91.3%. The expression patterns of either CK5 or ER between ADH and low grade DCIS were not significant ($p=0.265$, $p=0.061$). For CK 5: 11/13 (85%) ADH and 7/10 (70%) low grade DCIS cases were negative for CK5; all 22 UDH cases had diffuse staining (sensitivity 100%, specificity 91.3%). Interestingly, 2 ADH cases showed diffuse staining with CK5. The first case had cautery artifact and the second case showed both diffuse ER and CK5 staining. For PR: 5/13 (38%) ADH cases and 6/10 (60%) DCIS cases showed diffuse staining; none of the 22 UDH cases showed diffuse staining. Bcl-2 showed no statistical significance in expression pattern between UDH, ADH and DCIS ($p=0.815$).

Conclusions: While morphology remains the gold standard for the diagnosis of UDH, ADH and low grade DCIS, the combined use of ER with CK5 may increase the sensitivity of distinguishing ADH and low grade DCIS from UDH when compared to either stain alone. PR staining may also play a role in the differential diagnosis whereas Bcl-2 does not appear helpful.

212 GATA3 Is Frequently Expressed By Estrogen Receptor-Negative Breast Cancers: A Comparison of Two Commercially Available Monoclonal Antibodies

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Background: GATA-3 is a transcription factor demonstrated in gene expression profiling experiments to be highly expressed in breast cancer, where it was shown to correlate with estrogen receptor (ER)-positivity and favorable prognosis, and urothelial carcinoma. GATA-3 immunohistochemistry (IHC) has emerged as a key marker in the diagnosis of ER-positive breast cancer and urothelial carcinoma. It is at least as, if not more, sensitive than the traditional breast cancer markers ER and PR, with the advantage that it is not expressed in Müllerian tract tumors. It is nearly twice as sensitive as GCDFP-15 and mammaglobin. GATA-3 would be especially useful as a marker of ER-negative breast cancers, but, unfortunately, data are conflicting, with reported rates of GATA-3-positivity ranging from 12.5-71% (median 48%). Some of this variation may be due to choice of antibody clone.

Design: GATA3 IHC was performed on tissue microarrays of 200 ER-negative and 23 ER-positive breast cancers, using two monoclonal antibodies (L50-823, Biocare; HG3-31, Santa Cruz). Tumors were arrayed in triplicate. Extent (%) and intensity (0, 1+, 2+, 3+) of staining were assessed and an H-score (extent x intensity) calculated. An average H-score >5 was considered positive. Two-tailed Fisher's Exact Test and Wilcoxon matched-pairs signed-ranks test were used as appropriate, with $p<0.05$ considered significant.

Results: Strong GATA3 expression (average H-score 292) was detected in 100% of ER-positive breast cancers, regardless of clone. GATA3 expression was detected in 78.6% (average H-score 168) and 67.2% (average H-score 136) of ER-negative breast cancers with the L50-823 and HG3-31 clones, respectively. Both the frequency and extent of GATA3-positivity were greater with L50-823 than with HG3-31 ($p<0.0001$). Detailed expression data are presented in the Table.

GATA3 Expression in Breast Cancer Stratified by ER Status				
Clone	L50-823		HG3-31	
	% Positive	Average H-score (if positive)	% Positive	Average H-score (if positive)
ER-Negative	78.6 (n=196)	168	67.2 (n=192)	136
ER-Positive	100 (n=23)	292	100 (n=23)	292

Conclusions: Immunopositivity with monoclonal antibodies to GATA-3 is retained in the vast majority of ER-negative breast cancers, suggesting this as a key diagnostic marker in this tumor type, particularly useful in metastases. The L50-823 clone appears more sensitive and produces a more robust signal in this setting.

213 Mucocele-Like Tumors of the Breast: A Clinical Outcome and Histologic Analysis of 102 Cases From the Mayo Benign Breast Disease Cohort

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Background: Mucocele-like tumors (MLT) of the breast are classically defined as cyst-like lesions in which mucin distends lumens and extravasates through the lining epithelium into surrounding stroma. MLT are accompanied by a variety of epithelial proliferations, with an increased frequency of associated atypical ductal hyperplasia. There are no long term follow-up studies to assess whether MLT confer risk for subsequent development of breast cancer.

Design: Our benign breast disease (BBD) cohort is comprised of 13,434 women who underwent biopsy between 1967-2001. Archival slides were reviewed in a blinded, retrospective fashion. MLT were categorized as classic (with mucin extravasation), non-classic (without extravasation), or mechanically disrupted (MD). Cases were classified as MD when mucin was present at the edge of the biopsy or there was a defect in the tissue causing mucin extravasation. MLT were also analyzed for associated proliferative and atypical lesions (including flat epithelial atypia (FEA)), and for incidence of breast cancer in follow-up; the mean follow-up is 14.8 years.

Results: The cohort had 102 MLT (0.7%); 79 were classic, 9 were non-classic, and 14 were MD. Columnar epithelium, coarse calcifications, and abundant mucin were

common to MLT. At time of diagnosis, 42% of patients were greater than 55 years, 41.2% were 45-54 and 16.7% were less than 45. MLT were more often associated with atypical hyperplasia (AH) compared to the overall cohort (26% vs 5%, p<.0001). FEA was present in 8.8%. To date, 13 patients with MLT (12.7%) have developed breast cancer, with a mean interval between BBD biopsy and cancer diagnosis of 14.8 years. This breast cancer frequency is only slightly higher than the population expected rate (SIR 2.28, 95% CI 1.21-3.91), and not significantly different from women with proliferative breast lesions other than MLT, or columnar cell change. The mean age at cancer diagnosis was 65.8 years. Six cancers developed in the same breast as the MLT, 6 in the contralateral breast, and one bilaterally. Two had FEA in the BBD biopsy and 3 had AH. Four of 17 women under the age of 45 with MLT developed cancer, a frequency which is higher than the population expected rate (SIR 5.16, 95% CI 1.41-13.23).
Conclusions: MLT are a subset of columnar cell change which, for unclear reasons, are often associated with co-existing AH. Our data suggests that, overall, MLT do not appear to be unstable precursor lesions; however, MLT occurring in patients under age 45 may represent a high-risk subset.

214 Metastatic Breast Cancer: Can Bone Biopsy Be Used To Evaluate Hormone Receptor and HER2 Status?

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Background: Differences in hormone receptor (HR) and HER2 status between primary and metastatic breast cancer (BrCa) have been reported. Although bone is the preferential metastatic site, most data derives from studies of non-bone metastases, since decalcification is believed to impair immunoreactivity. We assessed the impact of decalcification in HR/HER2 status in bone metastases and therapeutic/prognostic implications of HR conversion.
Design: Material from bone (n=148) and visceral (n=120) BrCa metastases was clinicopathologically reviewed and compared with primary tumor for HR (immunostaining intensity/ positivity percentage) and HER2 status. In 34 primary BrCa, alongside with routine fixation, a parallel tissue sample was decalcified. Statistical analysis: McNemar's, Wilcoxon signed-rank, Chi-square tests and Cox proportional hazard model.
Results: Comparison of HR/HER2 expression between bone and visceral metastases

Figure 1. CHANGE IN HR/HER-2 STATUS BETWEEN PRIMARY TUMOR AND BONE/VISCERAL METASTASES

	ER conversion		PR conversion		HER2 conversion					
	+ → -	- → +	+ → -	- → +	3+ → 3+	3+ → -	3+ → 2+	- → -	- → 3+	- → 2+
BONE MTX	14/128 (10.9%)	1/11 (9%)	57/110 (51.8%)	2/110 (1.8%)	4/8 (50%)	1/8 (12.5%)	3/8 (37.5%)	78/82 (95.1%)	1/82 (1.2%)	3/82 (3.7%)
VISCERAL MTX	13/86 (15.1%)	1/86 (1.1%)	35/67 (52.2%)	2/67 (2.9%)	19/20 (79.1%)	1/20 (5.0%)	0 (0.0%)	41/47 (87.2%)	1/47 (2.2%)	5/47 (10.6%)

- There was a significant change of HR expression in bone (ER:p=0.001; PR:p<0.001) and visceral (ER: p=0.006; PR:p<0.001) metastases when compared to primary tumors.
- There were no significant differences in the proportion of ER (p=0.367) and PR (p=0.957) positive-to-negative conversion in bone and in visceral metastases.
- The proportion of PR conversion was significantly higher than ER both in bone (p<0.001) and visceral (p<0.001) metastases.

Regarding decalcified primary tumors, when considering only positive cases, there was no significant change in HR status, but there was a significant (ER: p=0.005; PR: p=0.001) reduction in the median intensity of the HR expression. Patients with positive-to-negative ER conversion had a worse survival (HR:1.95,p=0.03) compared with those that remained ER positive in the metastasis. PR conversion showed a similar trend (HR:0.90,p=0.156).
Conclusions: Bone is traditionally considered inadequate for HR/HER2 reevaluation but our results show that HR/HER2 immunoexpression is feasible and reliable in bone specimens, as it is not substantially influenced by decalcification. Importantly, careful evaluation of low intensity staining is mandatory and decalcification procedures must be standardized. Since bone may be the first and/or only BrCa metastatic site, and HR/HER2 conversion may impact treatment, validation of these results may significantly change clinical approach to metastatic BrCa.

215 Impact of 2013 ASCO/CAP HER2 Guideline Updates at an Academic Medical Center That Performs Primary HER2 FISH Testing: Increase in Equivocal Results and Utility of Reflex Immunohistochemistry

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Background: HER2 testing is recommended for all newly diagnosed invasive breast cancers and metastases to assess prognosis and suitability for targeted therapy. At our institution, we perform fluorescence in situ hybridization (FISH) as the primary assay to identify HER2 amplification. The 2013 ASCO/CAP guideline updates lowered the threshold for HER2 positivity and altered the equivocal category. The goal of this study was to evaluate the impact of these changes on the distribution of HER2 FISH status in breast cancer and, examine the utility of reflex HER2 immunohistochemistry (IHC) for FISH equivocal cases.
Design: We retrospectively identified all primary invasive breast cancers and metastases with HER2 FISH results during the 12 months before (10/2012–09/2013) and after (10/2013-09/2014) the HER2 guideline updates were implemented. All cases analyzed for HER2 via dual-probe FISH (PathVysion) were included. The distribution of HER2 FISH positive, equivocal, and negative cases was determined for each time period. Reflex HER2 IHC (clone 4B5) results were recorded for HER2 FISH equivocal cases. HER2 results were interpreted based on the guidelines that were in place at the time of testing.
Results: The distribution of HER2 FISH results before and after the guideline updates are listed in Table 1. There was a significant increase in the number of FISH equivocal results after the guideline updates (4.9% vs 1.4%, p= 0.0087) that was independent of specimen type (core vs. surgical, p= 1.0). Four of 5 FISH equivocal cases before the update would have been classified as positive using the new recommendations. Sixteen of 17 FISH equivocal cases after the updates had reflex HER2 IHC: 2/16 (12%) were positive, 11/16 (69%) remained equivocal and 3/16 (19%) were negative. Six FISH equivocal biopsy cases after the guideline updates had repeat FISH on the subsequent surgical specimen: 2 remained equivocal and 4 were negative.

	Before Updates (n= 355)	After Updates (n= 345)
Negative	311 (87.6%)	291 (84.3%)
Equivocal	5 (1.4%)	17 (4.9%)
Positive	38 (10.7%)	31 (8.9%)
Insufficient	1 (0.3%)	6 (1.7%)

Conclusions: Implementation of the 2013 ASCO/CAP HER2 guideline updates resulted in an increase in HER2 FISH equivocal results regardless of specimen type. Reflex IHC for FISH equivocal cases is of limited utility, but does assign HER2 positivity or negativity in a small percentage of cases.

216 MED12 Somatic Mutations in Fibroadenomas and Phyllodes Tumors of the Breast

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Background: The Mediator Complex Subunit 12 (*MED12*) gene encodes a protein that plays pivotal roles in the regulation of gene expression. Somatic mutations in *MED12* have been reported in several tumor types, including fibroadenomas (FAs) of the breast, which have been shown to harbor *MED12* exon 2 mutations in approximately 50% of cases. FAs and phyllodes tumors (PTs) likely constitute a spectrum of lesions with varying clinical behaviors. The aim of this study was to define whether PTs would be driven by *MED12* somatic mutations in a way akin to FAs.
Design: Samples of FAs (n=28), benign PTs (n=19), borderline PTs (n=15) and malignant PTs (n=7) from 57 patients were retrieved from the files of our Department. Lesions were classified according to the criteria set out in the current World Health Organization classification. Two pathologists with an interest in breast pathology reviewed all available slides and selected areas representative of the lesions and normal breast tissue for microdissection. DNA samples were extracted from each lesion and adjacent normal breast and subjected to Sanger sequencing using primers encompassing the *MED12* exon 2 coding sequence and the exon-intron boundaries. Forward and reverse sequence reactions were performed in duplicate. Sequencing results were analyzed using the DNAStar LaserGene software with the observers blinded to the diagnosis of each lesion.
Results: *MED12* exon 2 somatic mutations were found in 64%, 79%, 87% and 14% of FAs, benign PTs, borderline PTs and malignant PTs, respectively. Thirty somatic single nucleotide variants and 17 insertions and deletions affecting *MED12* were identified. Seven patients had multiple lesions; in 3 cases all lesions were found to harbor identical somatic *MED12* mutations, in 2 cases all lesions harbored *MED12* mutations but these varied from lesion to lesion, in 1 case of bilateral FA, one harbored a *MED12* mutation and the other was *MED12* wild-type, and in the last case both malignant PTs were *MED12* wild-type. Although we observed a step-wise increase in the frequency of *MED12* mutations from FAs to borderline PTs, malignant PTs significantly less frequently harbored *MED12* exon 2 somatic mutations (p<0.005, Fisher's exact test).
Conclusions: *MED12* exon 2 somatic mutations are found in the majority of FAs, benign and borderline PTs, but are significantly less common in malignant PTs. Although *MED12* is likely the driver of most FAs and PTs, our results suggest that genetic alterations other than *MED12* exon 2 mutations may constitute the driving genetic events of malignant PTs.

217 Strong Correlation of c-Myc Expression With High Grade Triple Negative Breast Ductal Cancers in African American Women

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Background: c-Myc is a proto-oncogene that regulates the expression of many target genes involved in cell proliferation and cell cycle regulation. Constitutive expression of mutant c-Myc can result in cell cycle dysregulation and uncontrolled cell proliferation. The significance of c-Myc expression and cell cycle dysregulation in breast carcinogenesis is poorly understood. The objective of our study was to correlate the immunohistochemical expression of c-Myc in the four major subtypes of breast carcinoma (Luminal A, Luminal B, HER2, and Triple Negative) in a population of 202 AA women with other clinicopathological factors including grade, stage, disease-free, and overall survival.
Design: Tissue microarrays (TMAs) were constructed from FFPE tumor blocks from primary ductal breast carcinomas in 202 AA women. Two separate 1 mm cores represented each case. The polymer-HRP system was utilized for immunostaining. Five micrometer sections are stained with a rabbit monoclonal antibody against c-Myc (EP121, Cell Marque, Rocklin, CA). The sections were evaluated for the intensity of nuclear reactivity (2-3) and the percentage of reactive cells; and an H-score was derived from the product of these measurements. Cases were categorized as having decreased (score ≤50) or increased (score >50) c-Myc expression. Bivariate analysis was done via c2 analysis and survivability data was calculated via the generation of Kaplan-Meier curves (SPSS v19). Statistical significance was assumed if $P < 0.05$.
Results: c-Myc expression was associated with ER negative (p=0.001), PR negative (p=0.031), triple negative (p<0.0001), and grade 3 (p=0.04). c-Myc expression was marginally associated with disease-free survival (p=0.085), but not overall survival.

Conclusions: Our study finding of enhanced c-Myc expression in high grade triple negative breast cancers supports the role of c-Myc breast carcinogenesis in AA women. A recent study has found that c-Myc stabilization by selective phosphorylation results in c-Myc with enhanced oncogenic activity due to inactivation of the axin 1 tumor suppressor gene, an important regulator of survival, growth, and stress pathways. Axin 1 may represent a possible therapeutic target for TNBC, a subtype without specific therapeutic options.

218 Nuclear Transcription Factor Sox10 Is Expressed in a Subset of Metastatic Triple Negative Breast Carcinomas

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Background: Sox10 is a nuclear transcription factor expressed primarily in tumors of neural crest origin, such as nerve sheath tumors and melanoma. However, we and others have previously demonstrated Sox10 labeling immunohistochemistry (IHC) in primary breast carcinomas, particularly in triple negative carcinomas (Human Pathol 2013;44:959-965). However, the expression of Sox10 in metastatic breast carcinoma has not been previously reported. Here, we investigate the labeling pattern of Sox10 in paired primary and metastatic breast carcinomas from 26 patients with known estrogen receptor (ER), progesterone receptor (PR) and Her2 status.

Design: Tissue microarrays (TMAs) containing samples of paired primary breast carcinoma (PBC) and surgically-resected metastatic breast carcinoma (MBC) from 26 patients (5-10 cores per tumor) were labeled with IHC for Sox10. The extent of nuclear Sox10 labeling was scored by percentage labeling as follows: 0 (0%), 1+ (1%-25%), 2+ (25%-50%), 3+ (50%-75%), and 4+ (>75%). Clinicopathologic data were recorded. The carcinomas were invasive ductal (n=23) or lobular (n=3), and consisted of 16 luminal A (ER+/PR+/Her2-), 8 triple negative (ER-/PR-/Her2-) and 2 Her2+ (ER-/PR-/Her2+) phenotypes. The metastases were solitary per patient and were located in the brain (n=11), lung/pleura (n=8), gastrointestinal tract (n=3), liver (n=2), ovary (n=1) and paraspinal soft tissue (n=1).

Results: Nuclear Sox10 labeling was seen in 3 (12%) MBC, all of which were Elston grade III, triple negative ductal carcinomas metastatic to the brain (n=2) or lung (n=1). Overall, 38% triple negative MBC were Sox10 positive, as compared to 0% luminal A and Her2 MBC (p=0.045). The Sox10 labeling intensity was 1+ weak in 2 cases, and 4+ moderate-strong in 1 case. The Sox10 labeling in these 3 MBC was increased (n=2) or stable (n=1) relative to the matched PBC, which displayed 0, 1+ and 2+ weak labeling. The remaining PBC were all Sox10 negative.

Conclusions: Sox10 labeling is seen in over a third of metastatic triple negative breast carcinomas, which like melanomas and nerve sheath tumors can also be S100 positive. The findings in this study support our previous observation that Sox10 is rarely seen in ER+ or Her2+ carcinomas. The differential diagnosis of a S100+, Sox10+, ER-high-grade malignancy of unknown origin should include both melanoma and poorly differentiated breast carcinoma. An immunopanel containing more specific markers, such as cytokeratin or p63 for carcinoma and HMB45 or melan A for melanoma, should be employed in this setting.

219 Micro-Intratumoral Heterogeneity and Multiple Tumor Cell Subtypes of HER2-Positive, ER-Positive Breast Cancers

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Background: HER2 and ER status are the most relevant predictive factors in determining treatment for breast cancer patients. Therefore, we compared HER2+/ER+ breast cancers cases with other breast cancer subtypes to investigate the relationship between HER2 protein expression, HER2 gene amplification, and ER protein expression and segment these cases in terms of intratumoural heterogeneity.

Design: Thirty-four selected HER2 IHC 3+ and 2+ breast cancer cases were examined using a brightfield HER2 and ER multiplex assay for the following pathological features: 1) HER2 phenotypic intratumoral heterogeneity, 2) HER2 genotypic intratumoral heterogeneity, 3) HER2 phenotypic and genotypic micro-intratumoral heterogeneity, and 4) HER2 and ER micro-intratumoral heterogeneity. The study cohort consisted of 11 HER2+/ER+, 9 HER2+/ER-, 12 HER2-/ER+, and 2 HER2-/ER- breast cancer cases.

Results: HER2 phenotypic intratumoral heterogeneity was defined as the identification of two or more HER2 protein expression levels (3+ to 0) within the same tumor tissue section. Phenotypic heterogeneity was observed mainly in HER2+/ER+ (7/11, 64%) and HER2-/ER+ (7/12, 58.3%) types and was also detected in HER2-/ER- breast cancer cases (2/2). Phenotypic intratumoral heterogeneity was observed in only one of nine HER2+/ER- cases (11%). HER2 genetic intratumoral heterogeneity was defined as the presence of tumor cells with a HER2/CEN17 ratio higher than 2.2 in 5–50% of the tumor cells. HER2 genetic heterogeneity was observed in HER2-/ER+ (5/12, 41.7%), HER2+/ER+ (2/11, 18.2%), and HER2-/ER- breast cancer cases (1/2). HER2 phenotypic and genotypic micro-intratumoral heterogeneity was defined as individual tumor cells containing amplified HER2 gene without HER2 overexpression. HER2 phenotypic and genotypic micro-tumoral heterogeneity was observed with HER2+/ER+ (8/11, 73%) and HER2+/ER- (3/9, 33%) types. Simultaneous analyses of HER2 and ER status demonstrated 4 types of tumor cell populations: 1) HER2 gene+/HER2 protein+/ER+; 2) HER2 gene+/HER2 protein+/ER-: 3) HER2 gene+/HER2 protein-/ER+; and 4) HER2 gene+/HER2 protein-/ER protein-.

Conclusions: Frequent HER2 phenotypic intratumoral heterogeneity and HER2 phenotypic and genotypic micro-intratumoral heterogeneity were observed in HER2+/ER+ breast cancer cases. Four distinct types of HER2 and ER micro-intratumoral heterogeneity were identified among HER2+/ER+ breast cancers. Those data suggest that the intratumoral heterogeneity of HER2 and ER status at individual cell levels may be an approach to segment breast cancer cases.

220 Intratumoural Heterogeneity Assessed By Biomarker Multiplexing and Cluster Analysis

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Background: Biomarker Multiplexing is an emerging technology to study expressions and co-localizations of multiple molecules of interest within a morphological context. We have previously described the development of a novel prototype of a micro-fluidic flow cell with an automated dye cycling strategy for repeated stain-image-bleach sequences, established cut offs and demonstrated good correlations with conventional immunohistochemistry (Clarke et.al., Histopathology, 64(2), 242-255, 2014). Quantification of signal intensity allows measuring biomarkers expression on a single cell and provides an opportunity to assess intratumoral heterogeneity.

Design: A total of 230,671 cells from 105 breast cancers were assessed for ER, HER2, Ki-67, and ALDH1. Signal intensity of each biomarker across all cores on the TMA was log transformed. Using an unsupervised K-median clustering algorithm, individual cells were grouped based on their biomarkers profiles.

Results: In an analysis based on 4 clusters, our analysis produced clusters that approximated to intrinsic molecular subtypes: two triple-negative clusters that are Ki67- or +, a Luminal A like cluster (ER+/HER2-) and HER2+ (HER2+/ER-). Individual cells within each cluster were mapped back to their corresponding TMA core demonstrating that almost all cores exhibit various levels of intratumoural biomarker heterogeneity. Interestingly, the extent of heterogeneity was significantly lower for cores consisting of HER2+/ER- cells, as 90-100% of cells within those cores are HER2+/ER-. Although individual cells didn't coexpress ER and HER2, there were three cases that consist of ER+ and HER2+ populations representing Luminal B tumors. Clustering into more than 4 groups yielded similar molecular profiles, with additional clusters further defining within each intrinsic subtype.

Conclusions: Biomarker multiplexing combined with cluster analysis is a powerful tool for quantifying biomarker expressions of individual cell in a tumour and for determining the extent of intratumoural biomarker heterogeneity. Analysis of a larger cohort will allow us to better understand the basis of heterogeneity within each subtype and potentially improve treatment planning and outcome.

221 Prediction of Local Recurrence in Ductal Carcinoma In Situ: Clinical Validation of DCIS Score

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Background: The Ontario DCIS population-based study identified women with pure DCIS from 1994-2003. We reported the DCIS Score (DS) clinical validation results (Rakovitch, SABCS 2014) showing prediction of risk of an ipsilateral local recurrence (LR). Here we evaluate the correlation between the DS and clinicopathologic features, and whether DS provides independent LR risk information in pts treated w/ breast conservative surgery (BCS).

Design: Pathology was centrally reviewed for: focality (multifocality=at least 2 foci of DCIS 5mm apart), size, grade, subtype, comedo necrosis & clear margins, (CM=no ink on tumor). The DS was obtained by quantitative RT-PCR. Cox modeling was used to determine the relationship between independent covariates, DS (hazard ratio (HR)/50 units) & LR.

Results: DCIS Score was evaluated in 718 women w/ DCIS tx with BCS alone (571 w/ CM). With a median follow-up of 9.4 years, 100 BCS alone w/ CM cases developed LR (44 DCIS, 57 invasive). In the primary analysis, among 571 pts treated by BCS alone with CM the continuous DS was significantly associated with LR in ER+ pts (HR 2.26; 95%CI 1.41,3.59; P=0.001) and in all pts (HR 2.15; 95%CI 1.43,3.22; P=<0.001). Univariable analyses showed that the DS, multifocality, size, histologic subtype, grade, and comedo necrosis were statistically significant predictors of LR risk. 10 yr Kaplan-Meier LR rates (95%CI) for all BCS pts alone w/ CM were: low risk DS 12.7% (9.5%, 16.9%); int risk DS 33.0% (23.6%, 44.8%) & high risk DS 27.8% (20.0%, 37.8%). In multivariable analysis, hazard ratios for factors associated with LR were: multifocality: 1.97 (95% CI 1.27-3.02); size: 2.07 (1.16-3.83); age<50: 1.75 (1.07-2.76); DS/50: 1.69 (1.08-2.62) & tumor subtype: solid vs. cribriform, 1.63 (0.97- 2.88). Patients with low DS and non-multifocal disease have a 9.7% risk of any local recurrence (4.3% for DCIS and 5.6% for invasive) at 10 yrs. DCIS Scores were widely distributed within each subgroup defined by the clinical and pathology characteristics. TheDS is only moderately correlated w/ grade, comedo necrosis and size (Spearman correlations <0.48).

Conclusions: For DCIS pts treated with BCS alone the DCIS Score, focality, tumor size and histologic subtype provide independent LR information. Pts with low DCIS score and non-multifocal disease may be considered for BCS alone.

222 Detection of Putative Stem-Cell Markers in Invasive Ductal Carcinoma of the Breast by Immunohistochemistry: Does It Improve Prognostic/Predictive Assessments?

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Background: Compelling experimental evidence from the last two decades supports the existence of a special type of neoplastic cell with stem-like features (so-called cancer stem cell/CSC) and their role in the pathophysiology and therapeutic resistance of mammary cancer. However, their practical role in human breast cancer remains undetermined.

Design: An immunohistochemistry panel of 10 putative CSC markers (CD34, c-kit, CD10, SOX-2, Oct 3/4, p63, CD24, CD44, CD133 and ESA/EPCAM) was applied to 74 cases of breast cancer, previously diagnosed, treated and followed in a Regional Cancer Center of Minas Gerais State, Brazil, from 2004 to 2006. Possible associations between CSC markers and classic variables of clinical/pathological relevance were investigated.

Results: The most frequently positive CSC markers were CD44, CD24, CD133 and ESA (the others were present in <15% of the cases). In addition, two CSC profiles were defined by double-labeling: CD24-/CD44+ and CD133+/ESA+ cells. The former was significantly associated to patients with more than 40 years-old, tumors of <2,0cm, early clinical stages ($p<0.05$), but increased death risk (4X; p=0.03, IC95%1.09-14.41). The latter was related to an increased in tumor relapse risk of 3.75X (p=0.04; IC95% 1.02-13.69). All CSC markers were more frequent in higher histologic grades.

Conclusions: Our results indicate that, despite being a low sensitive method, the detection of at least some of the putative CSC markers by immunohistochemistry may be of prognostic/predictive relevance, especially when used to complement traditional tools, such as the clinical staging.

223 How Do the Differences Between 2013 and 2007 ASCO/CAP HER2 Guidelines Affect Her2 Status in Breast Carcinomas?

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Background: The ASCO-CAP Her2 testing guidelines for breast cancer have been recently revised. Currently the positive cut off was lowered from >30% (2007 guidelines) to >10% (2013 guidelines) for IHC and from HER2/CEP17 ratio >2.2 (2007) to >2.0 for FISH (2013). We retrospectively evaluated the impact of 2013 guidelines on Her2 status of breast cancer as compared to 2007 guidelines with focus on equivocal and IHC/FISH discordant cases; and single or dual probe FISH assay.

Design: The HER2 data of invasive breast cancers diagnosed at Hospital of the University of Pennsylvania between 2009 and 2014 was reviewed according to 2007 and 2013 guidelines, respectively. At the time of the diagnosis, FDA-approved DAKO HercepTest and Vysis dual colored DNA probe (PathVysion) were used for IHC and FISH tests, respectively. Her2/Cep17 ratio and Her2/cell were recorded for FISH analysis.

Results: 1804 cases were evaluated with HercepTest, of these cases 615 had FISH which was routinely performed on all IHC equivocal (2+) and some negative and positive cases. Compared to 2007 guidelines, 2013 criteria increased Her2 positivity in breast cancers from 14.1% to 14.9% for IHC and 17.9% to 18.5% for FISH. The equivocal cases were decreased from 9.5% to 8.7% (from n171 to n156) for IHC, and 9.2% to 5.3% (from n57 to n33) for FISH. Discordant cases including both IHC-/FISH+ and IHC+/FISH- were increased from n14 to n22 and from n2 to n3, respectively with overall incidence of 4.1% (3.6% for IHC-/FISH+ and 0.5% for IHC+/FISH-) compared to 2.6% according to 2007 guidelines. 12 equivocal and 4 negative cases by Her2/cell criteria for single probe test were reclassified as positive by the ratio criteria for dual probe test in 2013 guidelines.

Conclusions: Compared the 2007 guidelines, application of the 2013 guidelines would lower Her2 equivocal cases for both IHC and FISH (8.7% and 5.3% respectively) while correspondently increase Her2 positive and negative cases with a risk of creating more discordant cases. However, the trade off for eliminating equivocal cases is a high discordant rate approaching to 5%, a QA standard set by CAP. Different from 2007 criteria, the 2013 Her2/Cep17 criteria is now a yes/no two-tier system for dual probe FISH assay, equivocal cases in dual probe assay would be expected to be lower (<26.7%) than that of single probe assay for which one can only apply the Her2/cell criteria inherited from the 2007 guidelines.

224 Significance of Percentage of 2+ IHC Positive Cells for the Prediction of HER2 Amplification Status in Breast Cancers

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Background: According to recent 2013 CAP/ASCO guideline, Her2 status is classified as equivocal when 2+ staining observed >10% of invasive tumor cells for which a reflex FISH is recommended. However, significance of 2+ cells ≤ 10% is unclear. The aim of this study is to investigate HER2 amplification by FISH in breast cancers with IHC 2+ staining and emphasize on the significance of percentage of IHC 2+ tumor cells, particularly the £10% category in final Her2 status.

Design: As a routine QA practice, database of both HER2 IHC and FISH test details was kept in the IHC lab of Hospital of the University of Pennsylvania. 180 cases of breast cancer with 2+ Her2 staining were identified in the data from 2009 to 2014. HercepTest was performed on Dako autostainer with Link 48 (Dako). The % of 2+ IHC stained invasive tumor cells were determined by visual estimation for % and intensity at the time of the diagnosis and categorized into three groups: £10%, 10-50% and >50% for this review. FISH was performed in all cases by using Vysis dual colored DNA probe (PathVysion) and data was scored according to 2013 ASCO/CAP criteria for this review.

Results: There were 22 cases (12.2%) in >50% IHC 2+ group, 102 (56.7%) in 10-50% group and 56 (31.1%) in £10% group. Overall, Her2 amplification by FISH was 25.5%. Her2 was amplified by FISH in 12 (54.5%) cases in >50% group; 26 (24.5%) in 10-50% group and 8 (14.3%) in £10% group; while not amplified in 9 (40.9%), 68 (66.7%) and 41 (73.2%), respectively, in each group. The remaining 16 cases (8.8%) were FISH equivocal.

Conclusions: Most of breast cancers (56.7%) with IHC 2+ Her2 expression contain moderate amount (10-50%) of 2+ positive cells. Although overall, 25% of all 2+ cases were Her2 amplified by FISH, Her2 amplification probability significantly increased to 54.5% in cases with >50% 2+ positive cells and decreased to 14% in 2+<10% cases. Currently the 2013 ASCO/CAP guidelines defined IHC 2+>10% as equivocal result but lacked clear classification for 2+£10%. Our data showed a relatively small but non-neglectable number (14%) of 2+ £10% cases was Her2 amplified by FISH which approaches to the overal incidence of Her2 overexpression (15-20%) in breast cancer. IHC Her2 result of 2+£10% should be regarded as equivocal and reflex FISH should be performed in cases with 2+£10% as recommended by the 2007 ASCO/CAP guidelines.

225 Correlation Between FOXP3 mRNA Expression, Regulatory T-Cells (Tregs) in Peripheral Blood and Tumor Tissue, and Outcome in Patients With Breast Carcinoma

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Background: *FOXP3* (Xp11.23) is a tumor suppressor gene involved in the pathogenesis of human neoplasms. Moreover, FOXP3 acts as a transcription factor that coordinates the immunosuppressive effect of T-regulatory cells (Tregs). In breast carcinoma (BC), the relationship between *FOXP3* mRNA expression and the content of Tregs within the tumor and in peripheral blood is unknown.

Design: We included 354 non-consecutive patients with BC. Tumors were classified immunohistochemically (IHC) into Luminal A (23%), Luminal B/HER2- (23%), Luminal B/HER2+ (16%), HER2+ (13%) and Triple Negative/Basal-Like (TN/BL) (25%). We evaluated *FOXP3* mRNA expression by qRT-PCR using TaqMan assays. Relative changes in gene expression were calculated as the fold-change (FC) by the $2^{-\Delta\Delta Ct}$ method. *PUM1* and *ACTB* were the reference genes. Normal breast tissue was used as control sample. FOXP3+/Tregs were quantified by IHC within the tumor and/or immediately adjacent stroma, in at least 3 high power fields (x400). $CD4^+CD25^{high}FOXP3^+$ Tregs were identified in peripheral blood obtained previously to surgical treatment (n=155) by Flow Cytometry, by the expression of CD4, CD25 and FOXP3. The results were correlated with clinicopathological factors and outcome.

Results: High median *FOXP3* mRNA expression was associated with Luminal A/B phenotypes, ≤3 positive lymph-nodes, Tregs tumor content and lower rate of recurrence (all $p\leq0.011$; Mann-Whitney U and Kruskal-Wallis tests). High tumor-infiltrating Tregs (≥15) were associated with grade 3, necrosis and TN/BL phenotype (all $p<0.02$; Chi-square or Fisher tests) and poorer survival (p=0.012; Kaplan-Meier, log-rank test). However, the number of $CD4^+CD25^{high}FOXP3^+$ Tregs in peripheral blood showed no correlation with any of the analyzed factors (all $p>0.05$).

Conclusions: Our results in a series of BC showed that increased *FOXP3* expression is associated with better prognostic factors and patients' outcome, in contrast to FOXP3+/Tregs tumor content. Nevertheless, $CD4^+CD25^{high}FOXP3^+$ Tregs blood count had no relevance.

Supported by Grants FIS 10/00082, AP-172/10, FCVI-HGUA (2010/PC-04 and 2011/PC-03).

226 Nuclear TAZ Protein Expression Associates With the Triple-Negative Phenotype in Breast Cancer

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Background: The Hippo signaling pathwayhas emerged as an important regulatory pathway in cancer. The final transducer effectors of this pathway are the oncoproteins TAZ and YAP1, transcriptional coactivators of target genes involved in cell proliferation and survival. TAZ has been previously reported to have a role in tumorigenesis in breast cancer, but detailed analyses in the different breast cancer phenotypes have not been conducted so far.

Design: We have analyzed TAZ expression by immunohistochemistry in a retrospective series of 640 invasive breast carcinomas, comprising estrogen/progesterone receptor positive, HER2 positive, and triple-negative tumors. In a subset of 51 frozen tumors, we analyzed the mRNA expression of TAZ by q-PCR, and we inspected the DNA methylation status of *TAZ*-encoding locus by using the Sequenom MassArray® MALDI-TOF platform. Amplification of *TAZ was* analyzed by FISH in 30 triple-negative tumors. Cox's regression analyses, which included the classical parameters, were performed to evaluate TAZ IHC expression as a prognostic biomarker in breast cancer.

Results: We found a striking association of TAZ nuclear expression with the triple-negative phenotype (60.5% TAZ positive, $p<0.001$), which was even strengthened when stratifying into the basal-like subtype (70.8% TAZ positive, $p<0.001$). We observed similar low methylation levels of *TAZ* locus both in triple-negative and estrogen/progesterone receptor positive tumors. Gene amplification was observed in 6.45% of triple-negative tumors analyzed. These data were further confirmed in a panel of breast cancer cell lines and in the TCGA dataset. Finally, patients with strong TAZ expression showed poorer clinical outcome regarding both recurrence and overall survival. Multivariate analysis showed that strong TAZ IHC expression had prognostic value for breast cancer specific survival, independent from classical factors (hazard ratio, 2.191; p=0.044).

Conclusions: TAZ enhanced expression in the triple-negative/basal phenotype is a relevant observation that contributes to gain a better understanding of the complex biology of this breast cancer type. Moreover, TAZ could represent a potential selective therapeutic target for triple-negative/basal breast cancer.
Supported by ISCIII PI13/02477, RD12/0036/0064 and RD12/0036/0017.

227 HER2 Chromogenic Dual In-Situ Hybridization in Cytology Cell Blocks of Breast Cancer Specimens: Is It Better Than FISH?

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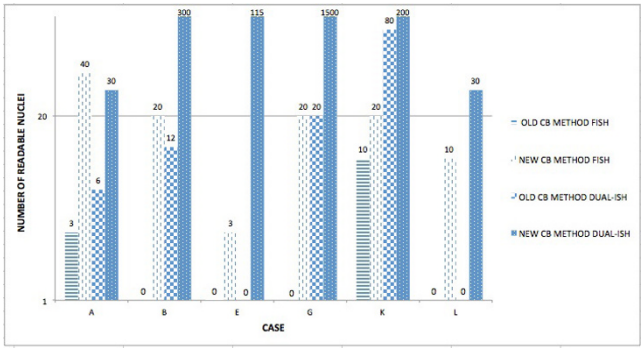
Background: Breast cancer (CA) metastases are often sampled by fine needle aspiration allowing the diagnosis of lesions using a minimally invasive technique. Cell blocks (CB) with adequate cellularity are crucial for ancillary studies. A recent study at our institution showed that an optimized CB protocol had the same or better cellularity and reliable ER and HER2 immunohistochemistry(IHC), while still complying with the ASCO/CAP guidelines. The aim of this study was to evaluate the HER2 status using chromogenic dual in-situ hybridization (Dual-ISH) in comparison with fluorescent in-situ hybridization (FISH) in paired CB using the prior established method (old method) and the new optimized CB method (new method).
Design: Twelve CB from 6 breast CA specimens with known 3+ HER2 IHC were prepared using the old and new methods. H&E slides were examined for cellularity. HER2 Dual-ISH (Ventana) and FISH (IQFISH pharmDx™, Dako) were done on all CB. New CB method included adding 5-10ml of 95% ETOH into formalin fixed cells and an additional centrifugation step. Dual-ISH slides were examined by light microscopy (60x) and FISH slides by fluorescent microscopy under oil (100x).
Results: All cases with detectable nuclear signal showed amplification (HER2/CEP17 range 3.5-17) and the ratio was similar for Dual-ISH and FISH. New CB method showed better cell aggregation without significant nuclear overlap. In 5 out of 6 cases (old CB method), < 20 nuclei (range 0-3) were detected with FISH and considered a failure. Results are summarized in Figure 1.



Conclusions: Accurate HER2 results are essential to guide therapy. The new CB method for Dual-ISH and FISH was more successful in identifying the amplification within CA cells due to improved cellularity and cell aggregation when compared to the old method. The advantage of Dual-ISH compared to FISH for CB is that CA cells are more easily identified at low power with better morphological detail, whereas scant and scattered cells may not be easily detected by FISH. Standard FISH protocols for tissue may be too harsh for scantier CB specimens leading to absence of cells. In this study of CB specimens, HER2 Dual-ISH proves to be of similar accuracy and an easier alternative for amplification detection compared to FISH.

228 Androgen Receptor Expression in Breast Cancer and Correlation With Pathological and Immunohistochemical Features

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Background: Androgen Receptor (AR) expression in breast cancer is associated with increasing age, lower tumor grade, smaller tumor size and ER, PR, Her2/neu expression. AR is potential therapeutic target for AR antagonists, especially in subset of triple negative AR positive tumors. The aim of this study is to evaluate AR expression in breast cancer patients in India and to correlate with histological features and ER, PR, Her2/neu expression.
Design: We planned to study AR expression in therapeutic subgroups, based on ER, PR, Her2/neu expression viz. (1) ER(+) - ER positive, Her2/neu negative, (2) Her2(+) - Her2/neu positive, irrespective of ER, PR expression, (3) TNBC - Triple Negative. We identified patients in each subgroup during period from January 2005 to December 2009. Slides and blocks were retrieved, slides were reviewed and tissue microarray (TMA) were prepared from representative tumor block. Immunohistochemical studies for AR, ER, PR and Her2/neu were performed on each TMA. Tumor was considered AR positive when ≥ 1% cells show nuclear expression. ASCO/CAP guidelines were used for ER, PR, Her2/neu evaluation. Clinical data was obtained from electronic medical records.
Results: Total number of cases selected were 109 in ER(+), 67 in Her2(+) and 92 in TNBC group. The frequency of AR expression in each subgroup in summarized below.

Subgroup	AR (+)	AR (-)
ER (+) (n=109)	45 (41%)	64 (59%)
Her2 (+) (n=67)	15 (22%)	52 (78%)
TNBC (n=92)	16 (17%)	76 (83%)

The correlation of AR expression with grade, tumor size and nodal status is as follow.

Parameters		ER (+)		Her2 (+)		TNBC	
		AR (+)	AR (-)	AR (+)	AR (-)	AR (+)	(AR (-)
Grade	I	1	1	0	0	0	0
	II	22	23	2	1	3	4
	III	22	40	13	15	13	72
T size	T1	16	14	2	15	5	11
	T2	27	46	12	29	10	63
	T3	1	4	1	8	1	2
Nodal Metastasis	Present	27	31	8	27	7	30
	Absent	18	32	7	25	9	46

Although AR expression was more frequent in ER (+) group in our series, we found fewer ER (+), AR (+) tumors (41%), when compared with western studies. However a subset of TNBC tumors (17%) is identified expressing AR. No significant association of AR expression with tumor grade, size and nodal status was found.
Conclusions: AR expression was found more commonly associated with ER expression. A subgroup of AR (+) TNBC tumors offers hope of targeted therapy.

229 Race and Incidence of Phyllodes Tumor: A Ten Year Retrospective Review

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Background: Phyllodes tumor of the breast is an uncommon neoplasm with a spectrum of behavior ranging from a benign fibroepithelial lesion to a sarcomatous entity with hematogenous metastases. Although historically described to be more common in women of Hispanic origin, few studies have examined the correlation between race and incidence. In this retrospective study, we reviewed the medical records of histologically confirmed phyllodes tumors diagnosed in our tertiary care institution over the past ten years, and characterize the incidence by race.
Design: Our study consists of 62 patients with benign, borderline, and malignant phyllodes tumors diagnosed between September 2004 and September 2014. As of 2013, our patient population of 183,744 consisted of 78,431 Hispanics (42.7%), 76,728 Whites (41.8%), 26,461 Blacks (14.4%), and 2,124 Other (1.1%). Data collected included patients' race, age, tumor grade, and tumor size.
Results: Of the 62 patients with phyllodes tumors in our study, there were 44 Hispanic patients (71.0%), 12 Blacks (19.4%), 5 Whites (8.1%), and one Asian (1.6%). Mean age at diagnosis was 43 in Hispanics, 39 in Blacks, and 41 in Whites. The correlation between race and histologic grade of the phyllodes tumors is illustrated in Table 1. Tumors were benign in 75%, borderline in 5%, and malignant in 20%. However, no significant correlation between race and malignant behavior was found (p = 0.938731), in contrast to previous reports in the literature.

Race	Benign Phyllodes Tumor	Borderline Phyllodes Tumor	Malignant Phyllodes Tumor
Hispanic	33 (33/44, 75.0%)	2 (2/44, 4.5%)	9 (9/44, 20.4%)
Black	10 (10/12, 83.3%)	0 (0/12, 0.00%)	2 (2/12, 16.7%)
White	3 (3/5, 60.0%)	1 (1/5, 20.0%)	1 (1/5, 20.0%)
Other (Asian)	1 (1/1, 100.0%)	0 (0/1, 0.00%)	0 (0/1, 0.00%)

Incidence of benign, borderline, and malignant phyllodes tumors by race.

Conclusions: Racial differences in the incidence of phyllodes tumors do exist. In this multi-institutional review, we found a significant disparity in the proportion of phyllodes tumors by race, with a substantially higher number of phyllodes tumors diagnosed in Hispanic women (p<0.00001). No correlation between race and tumor grade was found (p = 0.938731). Understanding the reason behind these findings will require further investigation into genetic predisposition, geographic influence, and socioeconomic factors.

230 The Relationship Between Quantitative HER2 Gene Expression By the 21-Gene RT-PCR Assay and Adjuvant Trastuzumab (H) Benefit in NCCTG (Alliance) N9831

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Background: There is considerable interest in developing HER2 testing criteria for adjuvant H. We used the 21-gene assay to examine the relationship of HER2 mRNA to benefit from H.

Design: N9831 compared adjuvant chemotherapy AC-T to concurrent chemotherapy-trastuzumab AC-TH in stage I-III HER2+ breast cancer. Recurrence Score (RS) and HER2 mRNA expression were determined by Onco*type* DX (neg<10.7, equiv 10.7 to <11.5, pos ³11.5 log2 expression units). Cox regression was used to assess the association of HER2 expression with H benefit for distant recurrence.

Results: Median follow-up: 7.4 yrs. Of 1940 total pts, 901 had consent and sufficient tissue. HER2 by RT-PCR was neg in 130 (14%), equiv in 85 (9%) and pos in 686 (76%) pts. Concordance between HER2 assessments was 95% for RT-PCR vs central IHC (>10% + cells = +), 91% for RT-PCR vs central FISH (³2.0 = pos) and 94% for central IHC vs central FISH. In the primary analysis, the association of HER2 expression with H benefit was marginally non-significant (P=0.057). In hormone receptor pos pts (local IHC) the association was significant (P=0.002). The association was nonlinear with the greatest estimated benefit at lower and higher HER2 mRNA expression levels. The observed treatment benefit in low HER2 pts was not due to imbalance between arms in RS and individual gene expression values.

Conclusions: Concordance among HER2 assessments by central IHC, FISH and RT-PCR was high. Association of HER2 mRNA expression with H benefit was marginally non-significant. A consistent benefit of trastuzumab irrespective of mHER2 levels was observed in the pts with either IHC+ or FISH+ tumors. Benefit was observed in pts with high HER2 by RT-PCR but also observed for the small groups of pts with negative results by quantitative RT-PCR or FISH. Plausible mechanisms for this observation will be discussed.

H benefit Cox HR (95% CI) by central HER2 status (adjusted for nodes)			
	Neg	Equivocal	Pos
IHC	0.31 (0.05,1.37)P=0.127	0.85 (0.27,2.31)P=0.763	0.49 (0.33,0.71)P
FISH	0.33 (0.09,0.93)P=0.034		0.54 (0.37,0.78)P
RT-PCR	0.31 (0.09,0.83)P=0.017	0.44 (0.09,1.59)P=0.217	0.55 (0.37,0.81)P

231 Surrogate Subtype (St Gallen 2013) and HER2 Positivity Are Strong Predictors of Breast and Axillary Response To Neoadjuvant Chemotherapy in Breast Cancer Patients

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Background: The aim of this study was to identify predictors of pathologic complete response (pCR) in breast and axilla after neoadjuvant chemotherapy in breast cancer.

Design: From 2009 to 2011, 226 patients received neoadjuvant therapy. Breast-pCR and axillary-pCR was defined as no invasive carcinoma: ypT0/ypTis and ypN0.

Variables analyzed on core biopsy: histologic grade, estrogen receptor(ER), progesterone receptor(PR), proliferative index(Ki67), HER2 and surrogate subtype (St Gallen 2013): Luminal A(LA), Luminal B(LB), Luminal B HER2 positive(LBHER2), HER2positive (HER2) and triple negative(TN). ER and PR were considered positive when ³10% of cells were stained. HER2 was determined according to ASCO-CAP guidelines(2007). Data were compared using Chi2 and Fisher's exact tests and multivariate analysis of variance, as appropriate. Statistical analysis was performed with SPSS version 15.

Results: pCR(ypT0/pTis ypN0) was achieved in 29 cases(13.3%). Taking into account only breast-pCR, 36 patients(15.9%) achieved ypT0 and 25(11.1%) ypTis. Breast-pCR rates were significantly different between surrogate subtypes: 3.6% in LA, 11.7% in LB, 29.4% in LBHER2, 60.6% in HER2 and 33.3% in TN. Of 133 patients with positive axillary nodes at diagnosis, 48(36%) became ypN0. Axillary-pCR rates were significantly different between surrogate subtypes: 12.5% in LA, 15.9% in LB, 77.3% in LBHER2, 66.7% in HER2 and 27.6% in TN.

On univariate analysis, factors associated with ypT0 were RE-, RP-, HER2+, Ki67>20%, grade and surrogate subtype (p<0.001). On multivariate analysis only surrogate subtype was found to be statistically significant(p<0.001). In the subgroup of patients with positive lymph node at diagnosis, HER2+, molecular subtype and breast-pCR were predictive of ypN0. On multivariate analysis, HER2+ was the only predictive factor of Axillary-pCR (p<0.001).

Conclusions: In this multivariate analysis, the strongest predictive factors of breast-pCR were surrogate subtype and HER2+ whereas HER2+ was the only positive predictor of axillary-pCR. Further molecular studies will help us to better select those patients who benefit most of neoadjuvant chemotherapy.

232 Genomic Abnormalities Correlated With Histologic Grade of Invasive Ductal Carcinoma of the Breast

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Background: Pathologic grade is one of the most important prognostic biomarkers in the patients with invasive breast cancer (IBC). The most clinically used Nottingham grade is based on three histologic features: tubule formation, nuclear pleomorphism and mitotic activity. However, little is known about the genomic basis of IBC histologic grading.

Design: We hypothesize that mutations in some breast cancer genes determine the histologic grade of invasive breast cancer. Whole genome sequencing data were analyzed in 1061 cases of invasive breast cancer. Specific genetic abnormalities were correlated with histologic grade of invasive ductal carcinoma. All sequencing data and corresponding pathology information were from The Cancer Genome Atlas (Invasive Breast Cancer, TCGA provisional) and were analyzed via cBioPortal bioinformatics tools.

Results: A total of 1323 mutated genes are identified in 976 IBC cases. Among the most frequently mutated genes are PIK3CA (316, 32.4%), TP53 (295, 30.2%), CDH1 (105, 10.8%), GATA3 (97, 9.9%) and MAP3K1 (70, 7.2%). Multiple mutations are found significantly associated with Nottingham histologic grade, including PIK3CA and TP53. The tumors with TP53 mutation are mostly histologically higher grade, but those with PIK3CA mutation often show a low to intermediate grade. The PIK3CA mutated tumors with a synchronous TP53 mutation show a significantly higher histologic grade compared with those without TP53 mutation. However, the TP53 mutated tumors with a synchronous PIK3CA mutation show a slightly lower histologic grade compared with those without a PIK3CA mutation. The results suggest that TP53 gene mutation alone may be sufficient to lead to a high histologic grade phenotype in IBC. While most tumors without TP53 mutation show a moderate histologic grade, those tumors with a PIK3CA mutation do show a significant lower histologic grade. These data suggest that a mutated PIK3CA gene may be an earlier event than TP53 mutation in breast cancer progression.

Conclusions: Mutations in TP53 and PIK3CA genes play important roles in defining histologic grade of invasive breast cancer.

233 Hedgehog Signaling in Immunophenotypes of Breast Carcinoma: A Gene Expression and Clinicalpathological Study

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Background: The Sonic Hedgehog (Hh) signaling is a key developmental regulatory pathway. It is recognized as a primary driver of oncogenesis in some types of human cancers. However, its involvement and prognostic relevance specifically in patients with breast carcinoma (BC) has not been studied extensively. Therefore, our aim was to analyze the expression of several Hh associated-genes in a clinical series of BC.

Design: FFPE samples of 186 BC were classified immunohistochemically into Luminal A and B/HER2- (43%), HER2+ (29%) or TN/BL (28%) phenotypes. qRT-PCR method was performed using *PUM1* as housekeeping. Relative mRNA expression of Hh genes (*GLI1*, *GLI2*, *GLI3*, *SMO*, *PTCH* and *BOC*) was determined using the $2^{\Delta\Delta CT}$ method and expressed as relative quantification (RQ) unit. The gene expression results were correlated with clinico-pathological factors and outcome of the patients.

Results: Significant association was found between several clinicopathological factors and gene expression, such as histological grade (for *GLI1*, *GLI2*, *GLI3* and *BOC*), vascular invasion (for *SMO* and *PTCH*), lymph-node status (for *GLI1* and *SMO*), necrosis (for *GLI1*, *GLI2*, *GLI3* and *BOC*), and phenotype (all p≤0.05, Kruskal-Wallis and Mann-Whitney Tests). Survival analysis showed that patients whose tumors expressed *GLI3* in low levels had increased recurrences, especially among those with TN/BL phenotype (p= 0.034). Overall survival was shorter in patients whose tumors expressed *GLI1* and *SMO,* predominantly in HER2 phenotype (p= 0.043 and p= 0.01, respectively). Interestingly, lymph-node negative patients with high tumor levels of *SMO* had poorer survival (p= 0.034) (Kaplan-Meier; log-rank test).

Conclusions: Several genes of the Hh pathway are expressed in all phenotypes of BC, supporting their involvement in this neoplasia. *GLI1*, *GLI3* and *SMO* expression stratified patients at risk of recurrence and/or death in TN/BL and HER2 phenotypes. Therefore, these facts make those genes as potential candidates for a therapy targets.

Supported by Grants FIS 10/00082 and FCVI-HGUA 2012/C-08.

234 Reflex Repeat HER2 Testing of Grade 3 Breast Carcinomas at Excision: Core Biopsy Parameters Including HER2 Immunohistochemistry & Dual In Situ Hybridization May Refine Case Selection

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Background: The updated ASCO/CAP guidelines (October 2013) for HER2 testing in breast cancer recommend repeat testing at excision of all grade 3 tumors that were HER2 negative on core needle biopsy (CNB). This has cost, time and workload implications. The aim of this study was to identify the rate of HER2 discordance between CNB and excision in this cohort of tumors.

Design: All Grade 3 HER2 negative cases diagnosed on CNB at a single institution over an 11 month period (Oct 2013 – Sept 2014) had reflex repeat HER2 testing at excision. Post-neoadjuvant therapy tumors were excluded. Histologic parameters including tumor subtype, grade and HER2 result for biopsy and excision were recorded. HER2 testing was performed in accordance with CAP guidelines using immunohistochemistry (IHC, Ventana 4B5) and dual in-situ DNA hybridisation (Dual ISH, Ventana INFORM) on cases with equivocal (2+) IHC results. All cases were dual reported by specialised breast pathologists.

Results: Diagnostic CNB and HER2 testing was performed on 520 breast cancers during this period.67 Grade 3 HER2 negative cases had repeat HER2 testing on excision. Tumor subtypes comprised invasive ductal carcinoma (n=59), invasive lobular carcinoma (n=5) and carcinoma with mixed ductal and lobular features (n=3). 22/67 were upgraded from grade 2 on CNB to grade 3 on excision. 63 cases (94%) showed concordant HER2 status on repeat testing on excision. In 4 cases (6%), the HER2 result changed from negative on CNB to positive on excision (table 1). 3 of these 4 cases showed equivocal HER2 IHC result on CNB and HER2 heterogeneity on excision. The 4th case was upgraded from grade 2 on CNB to Grade 3 on excision.

Case	Tumor subype	Grade		HER2 result: IHC score/Dual ISH	
		CNB	Excision	CNB	Excision (HER2:C17 ratio)
1	Ductal	2	3	2+/negative	2+/positive (2.42)
2	Ductal	2	3	1+/NA	2+/positive (3.43)
3	Lobular	3	3	2+/negative	2+/positive (2.49)
4	Ductal	3	3	2+/negative	2+/positive (2.30)

Conclusions: Our findings suggest that criteria for repeat HER2 testing at excision of grade 3 tumors that were HER2 negative on CNB may be refined to target tumors that were HER2 equivocal by IHC on CNB or were upgraded on excision, features indicating possible tumour heterogeneity. This could have significant cost and workload savings for busy laboratories.

235 Comparison of Margin Status Between Radioactive Seed Localized Versus Conventional Wire Localized Lumpectomy Specimens

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Background: Breast surgical margin status is one of the most important prognostic factors in terms of local recurrence for invasive carcinomas. The use of radioactive seed localization (RSL) has numerous benefits, including decreased patient discomfort, streamlined operating room scheduling, and increased potential use of intraoperative gross marginal evaluation. Despite these known benefits, few papers have looked at the resultant pathologic marginal status of these lumpectomy specimens, especially in regards to different definitions of close/positive margins. The goal of this project was to analyze and compare the marginal status of lumpectomy specimens removed by either RSL technique or conventional wire localization (CWL).

Design: Breast lumpectomy specimens for invasive cancers were analyzed and compared over a nine month span (Jan.-Sept. 2014). Data on specimen type, diagnosis including histologic grade, gross and microscopic surgical margin status, presence or absence of radioactive seed localization, and basic demographic information was retrospectively collected. Marginal status was defined as follows: close margin <2mm, very close margin <1mm, and positive margin = at ink.

Results: A total of 46 lumpectomies were analyzed for the margin status, both gross and microscopic. Comparisons of final microscopic margin status with presence of RSL or CWL was performed, see Table 1.

	Total cases	CWL	RSL
Mic. close margin (	12.3%	11.5%	13.9%
Mic. very close margin (	7.6%	7.1%	9.7%
Mic. at margin (0mm)	0.4%	0.6%	0%

Gross margin status on the specimens did not show significant differences for close margins (<2mm): 9% for conventional lumpectomies and 12.5% for RSL lumpectomies. Interestingly, there is a markedly increased percentage of close margins (<2mm) for invasive lobular carcinoma (25%), compared to invasive ductal carcinoma (10.4%). All percentages are reported per likelihood of having involved or close margins per one margin.

Conclusions: This study looked not only at direct involvement of inked surfaces, but also at close or very close margins, since surgeon/institutional preference varies in regards to the definition of a positive margin. As was shown before, no significant differences are seen for the marginal status for either RSL or CWL lumpectomy specimens taken out for invasive carcinoma. This project shows that this finding is valid at three typical cut offs used for margin assessment.

236 Reporting the Greatest Linear Extent of Ductal Carcinoma In Situ on Needle Core Biopsy

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Background: Regardless of size, ductal carcinoma in situ (DCIS) of the breast has a pathologic stage of pTis; however extent of DCIS correlates with local recurrence rates and the likelihood of close or positive margins. As a result, DCIS extent influences patient management and is therefore an important element in the College of American Pathologists tumor summary checklist for excision specimens. There are no recommendations regarding routine reporting of DCIS extent on needle biopsy and to our knowledge, no systematic studies have evaluated the impact of reporting this in needle biopsy material.

Design: Consecutive cases of DCIS, with or without microinvasion, performed or reviewed at our institution were identified by pathology report search over a 7 year period. The greatest linear extent of DCIS on needle core biopsy was compared to the estimated DCIS extent in the excision specimen. Cases with > 1mm invasion identified on excision were excluded.

Results: Of 241 total cases, there were 157 (65%) cases in which the DCIS extent on biopsy was smaller, 13 (5%) cases in which the sizes were equal, and 70 (29%) in which the size on biopsy was greater, including 30 (12%) cases with no residual tumor on excision. The mean extent of DCIS on excision was greater than that on core biopsy (16.0 vs 5.7 mm; Figure 1A); however the opposite was seen when only small tumors (£10 mm final size) were considered (4.5 vs. 3.6 mm; Figure 1B). There was very strong linear correlation between the size change (excision size minus biopsy size) and the final pathologic size (Figure 2).

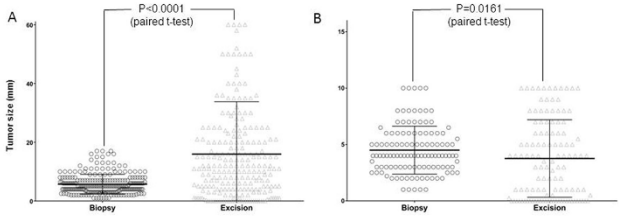

Figure 1: Comparison of biopsy and excision sized in all 241 cases of DCIS (A) and in 117 cases for DCIS in which final pathologic size is ≤ 10 mm (B).

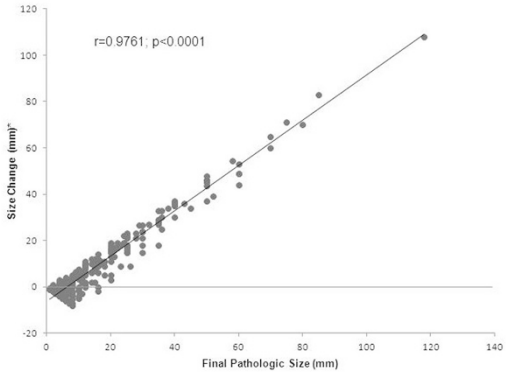

Figure 2: Relationship between the size change (*size on excision minus size on biopsy) and the final pathologic size (on the basis of the largest size measurement) in 241 cases of DCIS.

Conclusions: In a significant number of cases, the extent of DCIS on core biopsy was larger than that estimated in the excision. Due to the potential impact on clinical management, DCIS extent should be reported on all needle biopsy material, particularly in the setting of small tumors, when the biopsy specimen frequently represents the best overall approximation of DCIS size.

237 Increased Anti-Phospholipid Antibody Titers in Invasive Ductal Carcinoma Vs. Ductal Carcinoma In Situ: Implications for Surveillance

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Background: Understanding the role of anti-phospholipid (aPL) antibodies in tumor progression is important in breast cancer. Coagulopathic complications are common in patients with these cancers and patients with venous thromboembolism have a higher risk of mortality. Elevated aPL antibody titer has been observed clinically within a variety of malignant tumors. However, previous studies have not examined the relationship between aPL titers in pre-malignant verses invasive cancers. With the increase in DCIS diagnoses, questions regarding surveillance for malignancy are increasingly relevant. We propose that a higher aPL titer in invasive ductal carcinoma (IDC) vs DCIS may point to a potential utility monitoring DCIS patients.

Design: Patient serum samples with a diagnosis of DCIS (n=29) and IDC (n=47) were run on a Bio-Rad Autoimmune EIA using anti-cardiolipin IgM and anti-B2 glycoprotein I IgA test kits. Patients with titer levels greater than 11 anti-B2 glycoprotein I IgA units or greater than 20 anticardiolipin IgM units were considered positive for the respective antibody, according to criteria established by international consensus. All other results were considered negative.

Results: Anti-cardiolipin IgM titers were found to be positive in 0/29 DCIS patients and 8/47 IDC patients. Using Fisher's Exact test, the differences between these two categories is significant (P=0.021). However, anti-B2 glycoprotein I IgA titers were positive in 20/29 DCIS patients and 30/47 IDC patients. With 69% of DCIS and 64% of IDC patients positive for anti-B2 glycoproteinI IgA titers, the antibody is present in similar rates between these two groups.

Conclusions: There was a significant positive correlation between anti-cardiolipin IgM titers and IDC vs titers in DCIS. Further investigation is necessary to determine the causality of the relationship. One possibility is that the tumor cells may induce increased aPL antibody production, which induces other angiogenic factors and promotes tumor survival. Alternatively, high titers of aPL antibody may induce invasive transformation of pre-malignant lesions, pointing to a possible utility of routine aPL antibody testing in DCIS patients. No correlation was found between anti-B2 glycoprotein I IgA and IDC as compared to DCIS. However, both DCIS and IDC had high titers of anti-B2 glycoprotein I IgA compared to the incidence of aPL in the general population (3%). This raises the interesting question of whether anti-B2 glycoprotein I IgA levels may rise even earlier in the path to tumorigenesis than anti-cardiolipin IgM.

238 HER2 Positivity, High Mitotic Rate, and Tumor Grade Are Associated With Pathologic Complete Response To Neoadjuvant Therapy in Breast Cancer

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Background: Neoadjuvant chemotherapy is an effective tool in the management of patients with locally advanced breast cancer. However, breast cancers respond differently to neoadjuvant therapy. Pathologic complete response (pCR) is defined as no invasive carcinoma identified in the surgical resection specimen, and is strongly associated with improved patient outcome. To date, it is unclear which biomarkers can predict cancer pCR to neoadjuvant therapy. In this retrospective study, we analyzed the status of ER, PR, Ki-67 expression, Her2 amplification and tumor grade in pre-therapy biopsy samples to assess their ability to predict pCR.

Design: We searched 630 consecutive breast excisional specimens from Emory University Hospital archives and identified 70 patients received neoadjuvant therapy. The status of positive ER (1+, >1%), PR (1+, >1%) expression, Her-2 amplification (3+ by IHC or positive by FISH), proliferative index (raw Ki67 score), and tumor grade (high: Nottingham histologic grade 2 or 3 vs. low: Nottingham histologic grade 1) were evaluated and correlated with pCR. A Fisher's exact test or Student's t-test was performed for statistical analysis. A cut-off of a p-value <0.05 is used to determine significant correlation.

Results: Among the 70 cases of invasive carcinoma with neoadjuvant chemotherapy, 32 cases had pCR and 38 did not show pCR. Her2 positivity (59% in pCR group vs. 31% in non-pCR group, p = 0.027) and high Ki-67 score (p = .025) are significantly associated with pCR. Breast cancers with pCR are associated with decreased positivity of ER (34% in pCR vs. 58% in non-pCR, p = .057) and PR expression (24% in pCR vs. 42% in non-pCR, p = .082) and higher tumor grade (G2/G3 vs. G1; 97% vs 81%, p = .058), but these parameters were not significantly associated with pCR rate. When the 70 cases were classified into ER+/Her2-, Her2+ and ER-/Her2- subtypes, the Her2+ subtype was strongly associated with pCR rate (p = .0022).

Conclusions: Overall, Her2 positivity and high Ki67 score are significantly associated with high pCR rate in neoajuvant settings. Breast cancer with pCR shows lower proportion of positivity of ER and PR expression, and higher tumor grade, but the relationship is not statistically significant. This study highlights the significance of integrating the status of ER, PR, Her2, Ki67 and tumor grade into clinical decisions regarding neoadjuvant chemotherapy treatment.

239 Genomic, Transcriptomic and Prognostic Caracterisation of Medullary Breast Carcinoma in Comparision With Basal-Like Breast Carcinoma

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Background: Medullary breast carcinoma (MBC) is a rare entity (< 2 % of all invasive breast cancers). We previously showed that MBC was a triple-negative and a basal-like carcinoma but displayed distinguished genomic features from grade 3 non-medullary basal-like carcinoma (BLC). Here we tried to identify transcriptomic and pan-genomic alterations using massively paralell RNA sequencing from MBC and compare MBC prognosis with BLC prognosis.

Design: We retrospectively selected 19 MBC and 36 BLC with available frozen samples. We then performed a pan-genomic analysis on SNP array 6.0- Affymetrix. A transcriptomic analysis (Affymetrix 133 + 2) and tumor RNA sequencing (HiSeq 2000 Illumina; algorythms TopHat-fusion v2.0.4/defuse v0.6) were then performed on 19 and 6 MBC respectively. Overall survival (OS) and progression free survival (PFS) data were compared between MBC and BLC. Fluorescent In Situ Hybridation was used to confirm amplification.

Results: Unsupervised analysis of transcriptomic data identified two dinctinct groups, one inculded 75 % of MBC and the other 97 % of BLC. Statistical analysis (Welch test; fold change >3; $p\ value \leq 0.05$) showed significant overexpression of 92 genes and underexpression of 154 genes (GeneOntology: up-regulation of genes involved in hypoxia signaling pathway, MAPK, immune response, apoptosis and down-regulation of genes involved in cell adhseion). Tumor RNA sequencing allowed the identification of ten fusion transcripts, nine of them were validated with RT-PCR. One of the fusion involved *ETV6*. Pan-genomic profiles from MBC and BLC were similar but 23 losses of heterozygocity, 4 gains and 21 losses specific to MBC were identified (≥ 40 % of the cases). Three regions with amplifications, specific to MBC (16 % vs 6 % of BLC), were located on chromosome 10p, and the most frequent located in the surroundings of *ETV6* at 12p (26 % vs 17 % of BLC). Confirmation of *ETV6* amplification by FISH is being processed. Median follow-up was 79 months. OS and PFS (8.6 years vs 4.3 years, p = 0.0562) were longer in MBC group than BLC group.

Conclusions: We show that molecular alterations can define MBC as a genomic and a transcriptomic sub-category of basal-like carcinoma with the dysregulation of expression of genes involved in cell adhesion or immune response. We identify fusions which reccurrence among the whole MBC cohort is being verified by RT-PCR. Prognosis of MBC is better than BLC.

240 PASH: Does Identifying Whether PASH Is Focal or Diffuse on Core Biopsy Correlate With a PASH Nodule on Excision?

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Background: Pseudoangiomatous stromal hyperplasia (PASH) diagnosed on core needle biopsy (CNB) is generally excised. This may be appropriate if there is a mass lesion or PASH nodule. The CNB diagnosis of PASH as an incidental finding with other benign breast findings may lead to unnecessary excisional biopsy. This study categorized PASH in CNB as diffuse vs. focal to determine if this correlates with the presence of a PASH nodule which may necessitate surgical excision.

Design: A search for PASH cases on CNB from January 1, 1992 to December 31, 2012 identified 271 cases which were reviewed by two observers. On review, 216 were confirmed as PASH. These cases were subclassified as diffuse: PASH involving 2 adjacent lobules and focal: PASH in multiple non-contiguous lobules. Surgical follow up on the 217 cases was reviewed. Twenty one CNB with a concomitant pre-malignant or malignant diagnosis that would have led to surgical resection were excluded from surgical review. The surgical cases were classified as diffuse vs. focal PASH, using the same criteria. A PASH nodule on surgical excision was defined as a mass that could be measured grossly.

Results: The 216 PASH CNB were categorized as follows: 78 focal and 138 diffuse. Of these, 48 cases (22%) had surgical biopsies. 47 surgical excisions were reviewed (1 case not available) and 0/48 had a premalignant or malignant diagnosis. 75% (15/20) of focal PASH on core biopsy were focal on excision while 93% (25/27) of diffuse PASH cases were diffuse on excision. 2 (7%) cases of diffuse PASH were downgraded to focal on excision while 5 (25%) cases of focal PASH were upgraded to diffuse on excision. Fibroadenomas involved by PASH found on excision were considered concordant with a core diagnosis of diffuse (5 of the 25 cases) and discordant with a core diagnosis of focal (2 of the 5 cases). Of the remaining discordant cases (2 diffuse and 3 focal on core biopsy), sclerosing adenosis and stromal fibrosis were seen on excision. Using a Fisher's exact test the two-tailed P value is less than 0.0001 which is statistically significant.

Conclusions: When PASH is diffuse on CNB as defined by this study, diffuse PASH is present in the majority of excisions (93%). This finding in our study correlates with the presence of a mass lesion. Although the clinical and radiologic features may drive surgical resection, our findings support watchful waiting of focal PASH.

241 Assessing Mitotic Activity in Breast Spindle Cell Lesions Using Whole Slide Imaging: Comparison To Glass Slide Analysis

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Background: Mitotic activity in breast spindle cell lesions (BSCL) is important for accurate classification. These lesions are commonly seen in consultation, including digital consultation. It is unknown whether mitotic activity in BSCL can be accurately assessed using whole slide imaging (WSI). This study compares mitotic counts in BSCL in WSI vs glass slide analysis (GSA).

Design: 25 BSCL were selected from the files that included a variety of benign to malignant lesions. One slide per case was WSI scanned at 20x, 40x (Aperio) and set aside for GSA. For 25 cases two observers counted mitoses on 10 high power fields (HPF) on the WSI 20x and 40x scans and glass slide and recorded the time it took to complete each count . Areas that were counted were mapped. HPF on WSI was defined using the Aperio method. One observer counted contiguous fields and the second counted scattered fields on WSI. The mitotic counts and median time was averaged for both observers.

Results: Mitotic counts were highest for the GSA(98), followed by the 40x scan(74) and then the 20x scan (48). The differences were significant for WSI20x vs GSA (p value 0.001) and not significant for WSI40x vs GSA (p-value 0.033) indicating that 20x scanning for HPF analysis is the inferior method. The median mitotic count time for 10 HPF was: 1.32 min. (GSA), 5.77 min. (WSI40x) and 2 min. (WSI20x). The average total time for 25 slides was: 35.4 min (GSA), 151.1 min (WSI40x) and 52.8 min (WSI20x) indicating a four-fold increase in mitotic count time from GSA to WSI40x. The two field selection patterns yielded different mitotic counts (GSA: 116 vs 80), (WSI40x: 84 vs 64) (WSI20x: 67 vs 29) which was not statistically significant with a p-value of 0.2.

Conclusions: Mitotic activity can be assessed in BSCL using WSI with slides scanned at 40x but may not be as accurate as GSA. Slides scanned at 20x for HPF analysis may lead to inaccurate classification of BSCL in a digital consultation practice. Accuracy, and a four-fold increase in time to perform mitotic counts at WSI40x, should be taken into account when developing a breast digital consultation practice.

242 FOXA1 Expression in Invasive and In Situ Lobular Carcinoma

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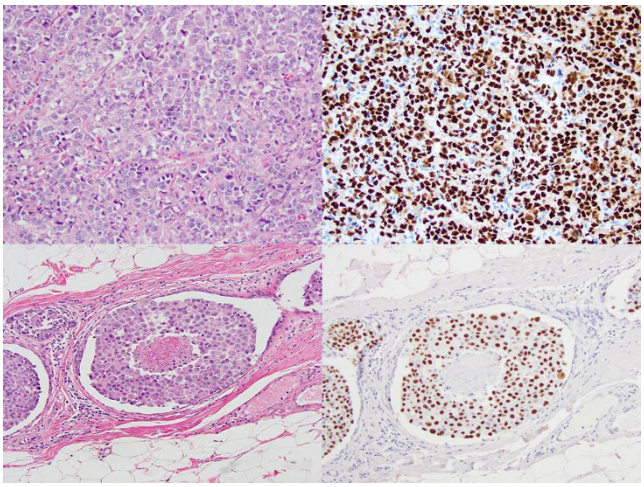
Background: *FOXA1* gene is located in human chromosome 14q and has a role in hormone-dependent cancers. FOXA1 is amplified in ER-positive breast cancer and it partly regulates AR activity. FOXA/AR-dependent WNT pathway activation leads to nuclear accumulation of beta-catenin and eventually HER3 induction. ER, AR, beta-catenin and HER3 levels were studied in invasive lobular carcinoma (ILC) and pleomorphic subtype of lobular neoplasia (PLN) by immunohistochemistry. In addition, mutation hotspots on 50 oncogenes and tumors suppressor genes were sequenced using Ion AmpliSeq Cancer Hostspot Panel v2 kit using Personalized Genome Machine.

Design: A total of 25 breast biopsies and resection specimens were retrieved from surgical pathology files. Fifteen out of 25 cases were ILC and 10 cases were pure PLN (40%). ILC group had 10 grade 2(67%) and 5 grade 3(33%) cancers. There was associated PLN in 14 ILC cases. Pleomorphic nuclear features were observed as a dominant component in one ILC case and focally in another. A combined ductal and lobular features was present in one of the invasive cancers. H-scores of ER, AR, HER3, beta-catenin and FOXA-1 expression were recorded separately for ILC, PLN and normal breast epithelium.

Results: All of the ILC and PLN cases expressed ER except one ILC and its associated PLN. The median H-score for ER expression in ILC and PLN was 290 (270-300) and 270 (175-285), respectively. The median of AR expression was 160 (140-250) for ILC and 170 (130-235) for PLN. The median H-score of FOXA1 expression was 230 (180-270) and 240 (190-285) for PLN and ILC.



There was no difference in ER, AR and FOXA1 expression between ILC and PLN. The median for beta-catenin and HER3 were 0 for both in-situ and invasive carcinoma. ER /FOXA1 and AR/FOXA1 expressions were positively correlated only in PLN group ($r>0.4$) ($p<0.05$). Marker expression was different in carcinoma and normal breast tissue ($p<0.001$). No pathogenic mutation was detected in the 207 sequenced regions on the 50 genes.

Conclusions: 1.ER and/or AR-positive ILC and PLN expressed FOXA1.
2.There was positive correlation in ER/FOXA1 and AR/FOXA1 expressions in PLN.
3.No association was found between hormone receptor and FOXA1 expression in ILC.
4.AR/FOXA/beta-catenin mediated HER3 induction was not observed in this small cohort of ILC.

243 Estrogen and Progesterone Receptors Expression in 2053 HER2 Negative Invasive Breast Cancer: Correlation With Ki-67, p53 and Tumor Grade

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Background: Hormone receptor (HR) positive breast cancer is a heterogeneous disease with a broad range of ER and PR expression. Studies suggest that ER+/PR- breast cancers are distinct from ER+/PR+ tumors. Assessment of ER and PR in most of the pathology laboratories is performed manually. Automated techniques such as image analysis (IA) provide a more objective assessment of biomarkers than manual estimation. We determined the relationship between ER, PR, Ki-67 index (KI), p53 and tumor grade using IA.

Design: We retrospectively analyzed 2053 cases of HR (+)/HER2 (-) invasive breast cancer from the database of the Department of Pathology, UT Southwestern Medical Center. Analysis of biomarkers was performed prospectively as part of a routine work-up of patients. Parallel sections from the same tumor block were utilized for immunohistochemistry using standard automated techniques. Prior to 2009, antibodies used were: ER (1D5, 1:200), PR (PgR, 1:1000), Ki-67 (MIB-1, 1:500), (Dako). Pre-diluted antibodies ER (SP1), PR (1E2), Ki-67 (30-9) and p53 (DO-7), (Ventana, Roche) after 2009. Criteria for positive staining for ER/PR and p53 is 1% and >10% positive staining respectively. The KI is categorized into low, intermediate and high (table 1).

Results: Of the 2053 cases, 1714 (83.5%) were ER+/PR+ and 339 (16.5%) were ER+/PR-. The median KI in ER+/PR+ vs. ER+/PR- was 15% and 29% respectively ($p<0.0001$).

	ER+/PR-	ER+/PR+	p value
Median ER expression (range)	81 (1-100)	97 (1-100)	
Grade			
1	56/254 (22.0%)	400/1432 (27.9%)	
2	101/254 (39.8%)	797/1432 (55.7%)	
3	97/254 (38.2%)	235/1432 (16.4%)	
Ki-67			
Low (≤14%)	111/339 (32.7%)	809/1714 (47.2%)	
Intermediate (15-30%)	68/339 (20.1%)	450/1714 (26.3%)	
High (>30%)	160/339 (47.2%)	455/1714 (26.5%)	
p53			
Negative (≤10%)	237/338 (70.1%)	1378/1670 (82.5%)	
Positive (>10%)	101/338 (29.9%)	292/1670 (17.5%)	

Conclusions: The ER+/PR- phenotype was associated with significantly lower ER expression, higher tumor grade, increased KI and p53 over-expression compared to the ER+/PR+ phenotype. Absence of PR may be a marker of aberrant growth factor signaling and may explain relative resistance to anti-hormonal therapy in this group.

244 The Clinical Utility of Alternative CEP17 Probes in Cases of Equivocal HER2 FISH Results

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Background: Anti-HER2 targeted therapy in combination with traditional chemotherapy regimens has been shown to improve outcome for breast cancer patients with HER2 positive tumors. ASCO/CAP recently issued new guidelines for HER2 testing in an attempt to capture as many breast cancer patients who are eligible for HER2 targeted therapy, and this included new cutoff points for HER2 FISH results as well as the use of alternative CEP17 probes for resolving equivocal HER2 FISH cases. The goals of this study are to determine the effect that the new guidelines have had on the number of HER2 equivocal cases and the clinical utility of alternative CEP17 probes as a reflex test for equivocal HER2 FISH results in clinical practice.

Design: Our data base was searched for consecutive cases of breast cancer where HER2 FISH was performed from October 2013 to September 2014. The HER2 copy number, CEP17 copy number, alternative CEP17 (TP53) copy number, HER2/CEP17 ratio, HER2/TP53 ratio, and amplification status based on both the 2007 and 2013 guidelines were recorded. PathVysion Kit (Her2neu/ CEP17 probes) and Vysis LSI TP53 probe were purchased from Abbott Molecular (Abbott Park, IL) and FISH was performed according the manufacturer's instruction.

Results: A total of 689 cases were identified. Three cases (3/689; <1%) were HER2 FISH equivocal by the 2007 guidelines whereas 20 cases (20/689; 3%) were equivocal using the 2013 guidelines. All 20 of these cases were reflexed to TP53 FISH resulting in 13 cases that were HER2 not amplified, 7 cases that were HER2 amplified and zero cases that were HER2 equivocal.

Conclusions: Equivocal HER2 results can make patient management decisions difficult when deciding if a patient will benefit from anti-HER2 targeted therapy. Since the implementation of the new HER2 guidelines, we have found about a 7 fold increase in the number of HER2 equivocal cases by FISH at our institution and use of an alternative CEP17 probe as a reflex test has resolved 100% of these cases. Therefore, our data supports routine use of alternative CEP17 probes as a reflex test for equivocal HER2 FISH especially with increased numbers of equivocal cases under the new ASCO/CAP guidelines.

245 CD44 Is Associated With FOXP3 Expression and Favorable Outcome in Patients with Breast Carcinoma

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Background: The CD44 transmembrane receptor mediates communication and adhesion between cells and extracellular-matrix. Recent research suggests that stimulation of CD44 induces FOXP3 expression in regulatory T-cells (Tregs), which are involved in the immune suppressive response. Moreover, *FOXP3* has been identified as a tumor suppressor gene that regulates tumor development. However, the relationship between CD44 and FOXP3 in breast cancer (BC) is unknown, and there is no conclusive data of their involvement in tumor progression. Our main objective was to study the association between *CD44* and *FOXP3* gene expression in BC specimens and cell lines, and to correlate the results with clinico-pathological variables and patients' outcome.

Design: We analyzed the mRNA expression of *CD44* and *FOXP3* by qRT-PCR in 264 BCs (38,6% Luminal, 28,4% HER2+, 33% Triple Negative/Basal-Like [TN/BL]). Relative quantification was calculated by the $2^{-\Delta\Delta Ct}$ method, using *PUM1* and *ACTB* as reference genes and normal mammary tissue as the calibrator sample. FOXP3+ Tregs were counted in 3 high power fields (x400) within the tumor and/or immediately adjacent stroma. Additionally, CD44 activation was performed by treatment with Hyaluronan in cell lines (MCF-7, BT474, SKBR3 and MDA-MB-468) representing BC subtypes, to study the changes in *FOXP3* and *CD44* mRNA expression.

Results: Lower*CD44* expression was seen in tumors with necrosis ($p=0.020$), vascular invasion ($p=0.011$) and grade 3 ($p=0.002$; Mann-Whitney U and Kruskal-Wallis tests). We found a positive correlation between *CD44* and *FOXP3* mRNA expression ($r=0.175$, $p=0.004$) as well as between *FOXP3* mRNA expression and Tregs content ($r=0.144$, $p=0.032$; Spearman's Rank Correlation). Moreover, activation of CD44 by Hyaluronan in cell lines showed an increase in *FOXP3* expression, confirming their positive correlation. Survival analysis revealed that patients with tumors showing low levels of *CD44* expression had increased recurrences ($p=0.039$; Kaplan-Meier; log-rank test).

Conclusions: Our findings in a clinical series of BC and *in vitro* indicate that *CD44* induces *FOXP3* expression. Furthermore, increased *CD44* expression is associated with good prognostic factors and better patients' outcome.

Supported by Grants FIS 10/00082, AP-172/10, FCVI-HGUA (2010/PC-04 and 2011/PC-03).

246 BRCA2 Mutation in Male Breast Cancer

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Background: Male breast cancer (MBC) is a rare entity and its management has been extrapolated from female counterpart. BRCA2 mutations confer increased risk of breast carcinoma, bilateral breast carcinoma (BBC) and other malignancies. BRCA2 founder mutations have been identified, including the previously reported c.156_157insAlu of portuguese origin.We aim to characterize a cohort of MBC with studied BRCA2 gene and analyze its clinicopathological features.

Design: We studied 25 non-related MBC cases with BRCA2 gene status evaluation. Medical charts and histological slides were reviewed. "Intrinsic" subtype was classified using immunohistochemical staining for hormonal receptors, HER2 and Ki67.

Results: Clinicopathological features and BRCA2 gene mutations presented in table 1 and 2.

Clinicopathological features	BRCA2 Gene	
	Non Mutated (n=15)	Mutated (n=10)
Median Age (years)	62	64.5
Family history, n(%)	4 (26.7)	7 (70)
Histological type, n(%) Invasive carcinoma, NOS Invasive lobular carcinoma Invasive papillary carcinoma	13 (86.7)1 (6.7)1 (6.7)	8 (80)1 (10)1 (10)
Histological grade, n(%) Well differentiated Moderately differentiated Poorly differentiated	2 (13.3)11 (73.3)2 (13.3)	06 (60)4 (40)
"Intrinsic" subtype Luminal A Luminal B (HER2-) Luminal B (HER2+) Triple negative	7 (46.7)6 (40)1 (6.7)0	7 (70)3 (30)00
BBC, n(%)	0	4 (40)
Other malignancies, n(%) Prostate carcinoma Gastric carcinoma Bladder carcinoma Colorectal carcinoma	1 (6.7)001 (6.7)	3 (30)1 (10)1 (10)0

No significant differences in age, family history, histological type and grade and "intrinsic" subtype were found between male patients with and without BRCA2 mutation. BBC and other malignancies were associated with BRCA2 mutation (p=0.02 and p=0.01). All patients presenting BBC and BRCA2 mutation also developed other neoplasias. Portuguese founder mutation (c.156_157insAlu) was encountered in two male patients (20%).

BRCA2 Mutation	MBC, n	BBC, n	Other malignancies, n
c.156_157insAlu	2	2	1
c.1786G>C/2	1	0	0
c.2808_2811del4	2	1	1
c.4380_4381del2	1	0	0
c.6468_6469del2	1	0	0
c.9098_9099insA	2	1	3
p.E475X	1	0	0

Conclusions: BRCA2 gene mutation status, although common in our MBC cohort (40%), has no effect on histological features. Bilateral breast carcinomas and other malignancies are more frequently found in male patients harboring BRCA2 mutations.

247 Clinicopathologic Features of Preinvasive and Microinvasive Breast Lesions in BRCA Germline Mutation Carriers

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Background: The clinicopathologic features of preinvasive and microinvasive breast lesions in *BRCA* germline mutation carriers have not been fully characterized. Here we sought to characterize the prevalence and clinicopathologic spectrum of preinvasive lesions and microinvasive carcinomas in patients with *BRCA* germline mutations.

Design: We searcheda database of 722 *BRCA* germline mutation carriers treated at our center from 1980 to 2013 for patients with a diagnosis of preinvasive (DCIS or ADH) or microinvasive (≤1 mm) breast disease and no evidence of ipsilateral invasive carcinoma. Clinical data were obtained, and all available slides were reviewed.

Results: A total of 69 patients (9.5%) were identified: 31 with a *BRCA1* mutation (BRCA1) and 38 with a *BRCA2* mutation (BRCA2). Patient average age was 44 years (range 24-79). Two patients were male (*BRCA2*). 33 patients had contralateral invasive carcinoma; 4 had bilateral DCIS. 9 BRCA1 patients also had a gynecologic malignancy. Most (47) patients underwent bilateral mastectomy; 13 bilateral lumpectomy or lumpectomy and contralateral mastectomy; 9 unilateral lumpectomy or mastectomy and had no contralateral disease on imaging. The index lesions were 60 DCIS (including 7 with microinvasion), 1 microinvasive carcinoma without DCIS or ADH, and 8 ADH. Imaging in most cases of DCIS +/- microinvasion showed mammographic calcifications (36) or a mass lesion (18). All ADH were incidental findings in risk-reducing mastectomy specimens. Slides of 38 index lesions were reviewed. BRCA1-DCIS had higher nuclear grade, greater lymphocytic infiltrate, and lower frequency of ER/PR positivity (50% vs. 95%) than BRCA2-DCIS (p=0.0007, 0.0046, 0.0244, respectively). Patients with ER(-)/PR(-) BRCA1-DCIS presented at an older average age (51.8 vs. 45.2) than ER(+)/PR(+) BRCA1-DCIS. Three of five ER(-)/PR(-) BRCA1-DCIS were available for immunohistochemical analysis, and all 3 were found to be positive for at least one of three basal markers (CK5/6, CK14 and/ or EGFR). Comedonecrosis was more frequently observed in BRCA1-DCIS whereas clear cell morphology was more frequent in BRCA2-DCIS, however these differences were not statistically significant.

Conclusions: DCIS in BRCA1 patients has clinical and histopathologic features distinct from those in BRCA2 patients. ER/PR negative lesions are more frequent in BRCA1 patients and often display a basal-like phenotype, whereas lesions in BRCA2 mutations carriers are preferentially ER/PR positive.

248 Characterization of Estrogen Receptor Negative/Progesterone Receptor Positive/(ER-/PR+) Breast Cancer (BC)

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Background: The value of PR testing in BC is a matter of ongoing debate. As an ER-dependent gene product, PR expression is theoretically a surrogate marker for a functional ER pathway. While ER-/PR+ BCs have a reported incidence of 1 to 4%, this subset of tumors is less well-defined, and it is unclear whether ER-/PR+ represents a distinct profile. The aim of this study was to characterize the pathologic features and prognostic significance of hormonal receptor (HR) positive BCs as defined by patterns of ER/PR expression.

Design: The tumor registry of the authors' institution was searched to identify BCs from 1997 to 2013. The clinicopathologic parameters of the primary BCs were recorded, along with therapeutic modalities and outcomes. The ER/PR status was examined following CAP guidelines. Analysis of relapse free survival (RFS) and disease specific survival (DSS) was performed by using the Kaplan-Meier method and the log-rank test.

Results: Of 5375 invasive BCs with known HR status, ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR- tumors were 3236 (60%), 680 (12.7%), 122 (2.3%) and 1337 (25%) cases, respectively. A significantly greater proportion of tumors with a high Nottingham histologic grade was found in ER-/PR+ BCs (53%) when compared to the ER+/PR- (36%) and ER+/PR+ (22%) phenotypes (p<0.0001). Further, the proportion of tumors with distant relapse were significantly lower in the ER+/PR+ subtype (7.6%) compared to ER+/PR- (13.2%; p<0.0001) and ER-/PR+ (14.8%; p=0.007) BCs. A significantly prolonged RFS was found in patients with ER+/PR+ BCs compared to those with ER+/PR- (p=0.002) or ER-/PR+ (p=0.0004) tumors. However, in patients who received hormonal therapy (HT), the ER+/PR+ BCs were associated a significantly prolonged RFS and DSS when compared to the ER+/PR- tumors (p=0.001 & 0.005, respectively), but did not significantly differ from ER-/PR+ BCs. No significant survival difference was found between the ER+/PR- and ER-/PR+ BCs in any group of patients analyzed.

Conclusions: ER-/PR+ BCs, albeit rare, exist as a distinct subset. Despite being mostly high grade, these tumors demonstrated a similar, if not higher, response rate to HT when compared to ER+/PR- BCs. The findings reflect the biologic machinery of PR acting as a marker for functional ER activation, thus strongly supporting the utility of routine PR testing in BC. While the mechanism for this unusual phenotype remains to be elucidated, a mutant ER may result in transcriptional activity thus leading to intact downstream signaling, a phenomenon previously reported in a cell line derived from an ER- BC.

249 Prognostic Factors in Advanced Breast Cancer (BC): Race and Receptor Status Are Significant After Development of Metastasis

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Background: The vast majority of BC mortality is due to distant metastases resistant to adjuvant therapies. Prognostic factors are well established in early-stage BC, but less well-defined in advanced disease. This study was aimed at determining the significant clinicopathological factors predicting survival outcomes in a subset of patients with advanced BC.

Design: The tumor registry of the authors' institution was searched to identify BC cases with associated distant metastasis from 1997 to 2010. Patients with metastatic disease at presentation and those with unknown receptor status were excluded. The clinicopathological factors analyzed included age, race, tumor type/size/grade, number of positive nodes, ER/PR/HER2 status and hormonal, radiation and adjuvant therapies received. Survival outcomes were estimated by the Kaplan-Meier method. The Log-rank test was used to compare groups.

Results: There were a total of 323 cases meeting the inclusion criteria in the cohort. By univariate analysis, race, tumor grade, ER, PR, and HER2 were significantly associated with overall survival (OS). Surprisingly, radiotherapy was associated with an adverse OS (p=0.04). Tumor grade and HER2 were significant factors for metastasis-free survival (MFS), while race, tumor grade, ER, PR, and HER2 were significantly associated with post-metastasis survival (PMS). Applying a multivariate Cox regression model revealed that tumor grade was a universal independent factor for survival outcomes and was the only factor significantly associated with MFS (p=0.01). Further, being Caucasians was advantageous for OS (p=0.024) and PMS (p=0.019). Similarly, HER2 positivity was a favorable factor for OS (p=0.013) and PMS (p=0.002), while PR positivity was significantly associated with a prolonged PMS (p=0.027). Interestingly, HER2-targeted therapy with Trastuzumab added significant benefit for OS (p=0.05) and PMS (p=0.05) only in the subset of patients with hormonal receptor positive (HR+/HER2+) tumors, but not in those with HR-/HER2+ disease.

Conclusions: Some of the previously established prognostic factors in early stage BCs, such as age, tumor size, and nodal status, may not entirely apply to patients with advanced BCs. While PR demonstrated a stronger prognostic value than ER, HER2 positivity was associated with a favorable outcome, more so if found after development of metastasis. The adverse effect of radiation seen in OS may reflect the recent observations that the risk of ischemic heart disease was significantly increased in women after radiotherapy for BC [N Engl J Med 368:987].

250 Primary Squamous Carcinoma of Breast – A Study of 27 Cases

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Background: This retrospective analysis sought to document on clinicopathological features of 27 cases of primary squamous carcinoma of breast and compare them with 28 metaplastic carcinomas showing squamous differentiation to highlight behavioral differences.

Design: Archives at our institute were analyzed for primary squamous carcinomas (PSC) of breast and 27 tumors where no demonstrable duct components were selected for further review. In addition 28 metaplastic carcinomas with only squamous differentiation (MCS) were retrieved along with case files for analysis.
Results: Of the 27 patients with PSC, 14 underwent modified radical mastectomy, 6 lumpectomy and 7 patients were initially misdiagnosed as benign cysts and underwent cyst excision. Of the PSC 18 were moderately differentiated, 8 poorly differentiated and one was a well differentiated squamous carcinoma. Interestingly though none of patients had a duct component, duct carcinoma in situ was identified in 4 patients and in two of them this in situ carcinoma showed squamous differentiation.
A comparison with MCS revealed that lymphovascular emboli were not seen in any PSC but were noted in 7/28 MCS. Nodal metastases was significantly more in MCS (12/27) as opposed to PSC(p -0.051). Most metaplastic carcinomas presented with 2 to 5cm sized tumors while most squamous carcinomas were 5 to 10 cm in size(p- 0.020). DCIS was seen in 4/27 PSC while 11/28 metaplastic carcinomas showed DCIS (p-0.040) While half of squamous carcinomas were circumscribed with a fibrous border they showed no intratumoral fibrosis, however most MCS showed infiltrating edges with dense intratumoral fibrosis.
Disease free survival (DFS) for MCS was 64% while overall survival (OAS) was 72.7% at 5 years. PSC had a DFS of 39.8% and OAS of 66.7%. The extent of squamous differentiation impacted DFS with best behavior for metaplastic carcinomas showing < 40% squamous differentiation and worse for pure squamous carcinomas (p – 0.043). Age had an impact on DFS in PSC with better survival in patients >50 years of age while no such impact was seen in MCS.
Conclusions: Primary squamous carcinoma of breast has unique histopathological features that make diffrentation from MCS easier. PSC however is an aggressive disease as compared to MCS especially those with <40% squamous differentiation.

251 Impact of Tissue Decalcification on Immunohistochemical Detection of Breast Carcinoma Markers

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Background: Application of an immunohistochemical (IHC) assay on decalcified tissues is frequently performed in an anatomic pathology laboratory. It has been documented that tissue decalcification (decal) may have a negative impact on an assay for certain antigens. In this study, we investigated the impact of decal on the detection of commonly used breast carcinoma markers using cultured breast cancer cell lines.
Design: Three breast cancer cell lines were obtained from the American Type Culture Collection. A set of IHC markers as listed in the Table were tested on each cell line on a cell block. Three cell lines collectively expressed all target markers. The cell pellets containing a mixture of the 3 cell lines were first fixed in 10% neutral buffered formalin for 8 hours and then decalcified in Decalcifier B (Fisher Healthcare, item #23245683) for the following durations: 0 minutes (no decalcification), 30 minutes (min), 60 minutes, 3 hours, 6 hours, 1 day, 3 days and 1 week. A tissue microarray (TMA) block containing these tissue/cell line cores with the different decal times was constructed. The aforementioned IHC markers were applied to the TMA sections using the Ventana Ultra staining platform. The staining intensity was recorded as strong (S), intermediate (I), or weak (W). The percentage of cells stained was recorded as 0, 1+ , 2+, 3+, or 4+ and compared to the sample with no decal.
Results: The results are summarized in the Table.

Markers/0 min	30 min	60 min	3 hours	6 hours	1 day	3 days	1 week
ER/4+,S	4+,S	4+,S	3+,S	3+,I	3+,I	3+,I	3+,I
PR/4+,S	4+,S	4+,S	4+,S	4+,S	4+,S	4+,S	4+,S
HER2/4+,S	4+,S	4+,S	3+,S	2+,I	2+,I	2+,W	2+,W
GATA3/4+,S	3+,S	3+,I	3+,I	3+,I	3+,I	3+,I	3+,I
GCDFP15/4+,S	4+,S	3+,S	3+,S	3+,S	3+,S	3+,S	3+,S
Ki67/4+,S	3+,S	2+,I	2+,I	1+,I	1+,I	1+,I	N/D
TFF1/2+,S	1+,S	1+,S	1+,S	1+,S	1+,S	1+,S	1+,S
TFF3/2+,S	2+,S	2+,S	2+,S	2+,S	2+,S	2+,S	2+,S
p53/4+,S	3+,S	2+,I	2+,I	2+,I	2+,I	2+,I	2+,I
CK7/4+,S	3+,S	3+,S	3+,S	3+,S	3+,S	3+,S	3+,S

Conclusions: These data demonstrate that tissue decal 1) has a significant negative impact on HER2 detection and on Ki67 and p53 detection after 60 min and 30 min of decal, respectively; 2) has no negative impact on ER detection following 60 min of decal and minimal impact following 1 week's decal; 3) has no negative impact on the detection of PR, GATA3, GCDFP15, CK7, TFF1, and TFF3 following 1 week's decal.

252 Kallikrein-Related Peptidase 6 Regulates miR-34a and miRNA-Mediated Networks To Affect Oncogenic Cell Cycle Pathways in Breast Cancer

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Background: The human kallikrein-related peptidases are a 15-member family of serine proteases that are reported to be differentially regulated in many cancers. miRNAs are single stranded RNA molecules that regulate the expression of their target genes.
Design: In order to understand the mechanism by which *KLK6* can affect breast cancer pathogenesis, we examined the effect of KLK6 reconstitution on miRNA expression, followed by bioinformatics analysis of the predicted target of the significantly dysregulated miRNAs.
Results: KLK6 over-expression resulted in up and down-regulation of a number of miRNAs in breast cancer cell lines. miR-34a and miR-888 were among the significantly down-regulated miRNAs, whereas miR-146a and miR-106a were up-regulated. Target prediction, pathway analysis, and experimental confirmation identified convergent miRNA networks targeting the MYC and MAPK and other signaling pathways. KLK6 overexpression resulted in down-regulation of miRNAs such as miR-34a, which led to up-regulation of their targets, including MAPK signaling genes (MAPK13, MAP2K1), CDKN1C and other cell cycle genes, and the oncogene MYC. We identified differential KLK6 expression in the molecular subtypes of breast cancer. Our data demonstrates breast cancer molecular subtype variations of KLK6 expression (in Her-2, Basal-like, Luminal A and Luminal B), with associated alterations of downstream miRNAs such as miR-34a, and their signaling targets in these subtypes. Long term patient survival outcomes were significantly reduced with altered KLK6 expression and high (KLK6+MYC+CDKN1C)-MUC1 scores.
Conclusions: We provide evidence that KLK6 dysregulation in breast subtypes affects MYC expression as well as MAPK signaling pathway genes through miRNAs networks, mediated at least partially through an alteration of the miRNA biogenesis machinery with differential patient outcomes in these groups.

253 Are Radial Scars at Core Biopsy High Risk Lesions? A 10-Year Single Institution Study and Literature Review

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Background: The core biopsy literature considers radial scars (RS) a high risk lesion based on studies showing a high chance of finding associated malignancy on excisional biopsy. These studies have significant shortcomings, and recommendations for or against surgical excision are variable. With the advent of a new imaging technique known as tomosynthesis (3D mammography), studies have shown at least a 2-fold increase in the detection of RS. Thus, determining the true risk potential of these benign lesions is becoming more critical.
Design: We identified 15 published studies from 1999-2014 that analyzed the rate at which benign RS on core needle biopsy (CNB) are upgraded after surgical excision. All of the studies had at least one of these confounding factors: [1] no radiologic-pathologic concordance of CNB results and/or including discordant cases in their series, [2] including CNBs with RS plus another risk lesion (e.g. ADH), [3] including risk lesions (e.g. ADH) as upgrades, [4] no comment on the distance of the upgraded lesion from the biopsy site, leaving the possibility that the upgraded lesion was unrelated to the RS, and [5] no histologic review of biopsies by a breast pathologist. To date, there is no study of RS that controls for all of these variables. Therefore, with IRB approval, we performed a computer-based search of all breast CNB performed at UNC from July 1, 2004 – June 30, 2014. All of the above factors were addressed in our study.
Results: Adjusting for the aforementioned confounding factors, 53 RS were followed up with surgical excision over the 10-year period. No concordant cases were upgraded on excision (0/53). Our literature review identified a total of 831 RS, of which 38 (4.6%) had reported upgrades to malignancy on excision. However, after applying all of the above criteria to each study, only 3 cases documented upgrades to malignancy, with the distance of the malignancy to the biopsy site ranging from 3 mm to 1 cm. By adding our study to the previously published cases, we find an upgrade rate to malignancy of <0.5% (3/884).
Conclusions: Our institutional data and a thorough review of the literature suggest that benign RS, when concordant and adequately sampled, do not pose a "high risk" of discovering malignancy on excisional biopsy. Since discovery of these benign lesions is likely to increase as tomosynthesis becomes more widely adopted, eliminating the label "high risk lesion" for radial scars and strict adherence to radiology-pathology concordance in each case can prevent a large number of unnecessary surgical excisions.

254 Splicing Variants of Androgen Receptor in Breast Cancer

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Background: The androgen receptor (AR) and its pathway have been implicated in tumorigenesis and progression of breast cancer. Recent studies identified at least 15 splicing variants of AR (AR-Vs). Structurally, AR-Vs have insertions of cryptic exons downstream of the exons that encode the DNA-binding domain or deletions of the exons encoding the ligand-binding domain, resulting in a disrupted AR open reading frame and expression of ligand-binding-domain-truncated AR proteins. In prostate cancer, some of the AR-Vs are associated with aggressive disease. The AR-V expression in human breast cancer specimens has not been studied. We aimed at studying the expression of AR-Vs in breast cancer specimens and present the data on AR-V1, AR-V7, AR8, and AR^{v567es}, particularly in AR-positive triple negative breast cancer (TNBC).
Design: Nine cases of TNBC were tested in this preliminary study. Cancer areas were macrodissected from formalin fixed paraffin embedded sections with total RNA extracted by using the PureLink® FFPE RNA Isolation Kit (Invitrogen). Reverse-transcription was performed by using the SuperScript® III Reverse Transcriptase Kit (Invitrogen). AR-V expression was presented as cycle number difference to housekeeping gene (delta CT) for real-time PCR or as absolute copy number for digital PCR.
Results: AR-V7 was expressed in all 9 cases with delta CT values varied from 0.14 to 7.84 with mean as 3.75. AR-V1 and AR-V4 were expressed in 4 of 9 cases (overlapping in 2 cases) with copy numbers ranging from 7.4 to 36. AR8 and AR^{v567es} were expressed in 2 of 9 cases with copy numbers ranging from 10.8 to 52.

Conclusions: We report expression of different patterns of AR-V in triple negative breast cancer. These data are encouraging and warrant a study in a larger cohort of cases. AR maybe useful for both prognosis and targeted therapy in triple negative breast carcinoma.

255 Impact of 2013 College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) Guideline Recommendations on Human Epidermal Growth Factor Receptor 2 (HER2) Fluorescent In-Situ Hybridization (FISH) Testing in Breast Cancer – Retrospective Study of 709 Cases

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Background: The CAP/ASCO guideline recommendations for HER2 FISH testing in breast cancer were updated in 2013. The guidelines recommend that a tumor with a dual probe HER2/CEP17 ratio ≥ 2.0 or an average HER2 copy number ≥ 6.0/cell be considered HER2 positive (HER2+) and a dual probe HER2/CEP17 ratio < 2.0 with average HER2 copy number ≥ 4 and <6/cell be considered HER2 equivocal (HER2e). The new guideline also eliminates the dual probe HER2/CEP17 ratio equivocal category. The aim of this study is to investigate the impact of the 2013 guideline recommendations on classification of HER2 FISH results.

Design: Breast cancer cases from 09/2009-08/2014 that underwent reflex HER2 FISH testing based on 2+positive HER2 immunohistochemistry results were included in the study. HER2 FISH reports were reviewed and the cases were classified as HER2 negative (HER2-), HER2e and HER2+ using 2007 and 2013 CAP/ASCO guideline recommendations. The HER2 FISH results based on 2007 and 2013 guidelines were tabulated for comparison.

Results: A total of 709 cases were included in the study. HER2 FISH classification of 665 (93.8%) cases remained unchanged using both 2007 and 2013 guidelines. The 2013 guidelines classified 22 (3.1%) less cases as HER2-, 12 (1.7%) more cases as HER2+ and 10 (1.4%) more cases as HER2e. Of the 12 cases reclassified as HER2+, 7 cases had dual probe HER2/CEP17 ratio ≥ 2 and ≤ 2.2 and other 5 cases had HER2 copy number ≥ 6/cell. Reclassification of 27 HER- cases as HER2e would have triggered further investigation. The frequency of chromosome 17 polysomy (average CEP17 copy numbers ≥ 3) was higher in the reclassified cases (20/39) as compared to the cases(5/665) with similar classification with old and new guidelines.

	2007 HER2 -	2007 HER2 equivocal	2007 HER2 +	Total
2013 HER2-	600	5	0	605
2013 HER2 equivocal	27	13	0	40
2013 HER2+	0	12	52	64
Total	627	30	52	709

Conclusions: The 2013 CAP/ASCO guideline recommendations for HER2 FISH testing classified more breast cancer cases as HER2+ and HER2e and fewer cases as HER2-. Cases reclassified as HER2+ would have been eligible for HER2 trageted therapy. Breast cancers with chromosome 17 polysomy were more likely to be reclassified using the new guidelines.

256 Analysis of Accuracy of ER and HER2 Expression By Immunohistochemistry and RNA-Sequencing on Core Cut Biopsies of Invasive Breast Cancer

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Background: RNA-sequencing (RNA-seq) is an alternative method for the analysis of gene expression, and may be used to overcome difficulties that are inherent to RNA microarray technology. Also, RNA-seq has a higher dynamic range compared to conventional gene expression techniques and therefore may be advantageous for the quantification of gene expression. We have compared routine immunohistochemistry (IHC) results obtained on breast core biopsies with gene expression by RNA-seq, and analyzed discrepant results.

Design: Fresh frozen tumor tissue was collected from core cut biopsies of 267 cases of primary invasive breast cancer, after obtaining informed consent. RNA-seq was performed using generation sequencing (NGS). Routine IHC was performed on the core biopsies for ER, PgR, and HER2 expression on a fully automated system (Ventana), and validated primary antibodies. In case of HER2 2+ results, HER2 amplification was determined by CISH. Intrinsic subtypes were calculated according to clustering for RNA-seq. Discrepant cases were subjected to further analysis.

Results: A high concordance rate between IHC and RNA-seq was observed for hormone receptor- (HR-) positivity, with a calculated accuracy of 94.7%. Discrepant cases that were HR-positive by IHC (irrespective of HER2-status) fell into HR-negative clusters by RNA-seq in 7.5%, but only 0.5% HR-negative cases were clustered as HR-positive by RNA-seq. With HER2, the accuracy rate for RNA-seq was calculated as 91.2%, with 4,9% false positives and 4,5% false negatives. For triple-negative cases by IHC, the calculated concordance (accuracy) was 95.8% when compared with the respective cluster as determined by RNA-seq. Further analysis that included the IHC from resection specimens showed that some but not all of the discrepancies could be explained by heterogenous antigen expression or by low hormone receptor positivity in IHC in cases that were non-luminal by RNA-seq. Further possible sources of discrepancies could include non-representative tissue for RNA-seq.

Conclusions: RNA-seq appears to be a reliable method for predicting positive ER- and HER2-status, with an overall accuracy rate of 93,6% in the whole cases series. Discrepancies occurred due to low hormone receptor expression on IHC, because of HER2 heterogeneity, or sampling issues.

257 Biomarker Profile in Metastatic Breast Cancer

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Background: It is recommended that breast biomarker testing be performed on all metastatic breast cancer (MBC). The aim of this study was to compare the biomarker profile of MBC according to metastatic site with established benchmarks in primary breast cancer (BC).

Design: After ethics review, we searched our files for MBC on which biomarkers were tested between 2001 and 2014. Age, type of procedure and metastatic site were recorded. Only patients with clinically MBC were included, especially amongst those with triple negative (TN) MBC or unusual metastatic site. We used the following cut-offs: for hormone receptor positive ³1%; for HER2 positive either 3+ staining in >10% by IHC or ³2 HER2/CEP17 ratio or ≥6 HER2 copies by ISH. Using the surrogate definitions of intrinsic subtypes of BC according to St Gallen International Expert Consensus the metastases were grouped into luminal A, luminal B, HER2 overexpression and basal-like (Ann Oncol (2013) 24 (9): 2206-2223).

Results: We found 343 MBC from 330 women (11 had 2, one had 3 different metastases) at a median age of 62 (mean 61, range 21-92). The most common sites were liver (27%), brain (17%), lung (13%), bone (13%), skin (13%), followed by soft tissue (4%), ovary (4%), pleura, mediastinum, adrenal, omentum, peritoneum, pericardium, dura, colon, vagina, parotid, and submandibular gland. Most specimens were biopsies (89%); 38 were excisions and 2 FNAs. ER testing was performed on 321 (93%) specimens, PR on 308 (90%) and HER2 on 311 (91%); all 3 biomarkers were available for 286 (83%) tumors.

				INTRINSIC SUBTYPES AS PER ST. GALLEN CONSENSUS %			
SITE (n)	ER+	PR+	HER2+	LUMINAL A	LUMINAL B*	HER2 OVER-EXPRESSION	BASAL-LIKE
TOTAL (343)	77	49	25	32	44	9.5	14.5
LIVER (93)	82	56	24	36	47	7	10
BRAIN (57)	51	23	57	2	43	27.5	27.5
LUNG (45)	81	57.5	31	34	48.5	6	11.5
BONE (44)	80	54	10	42.5	42.5	2.5	12.5
SKIN & ST (58)	83	60	13	40	40	5	15
OTHER (46)	84	66	12	38	44	5	13

Table 1: Summary of biomarker expression in MBC; *defined as ER+, PR

Conclusions: HER2+ and luminal B BC is more frequent in metastatic than in primary setting; the opposite is true for luminal A and PR+ BC. Brain metastases are more likely to be HER2+ or TN. Bone is a very uncommon metastatic site for HER2+ BC. Laboratories should account for these variations when analyzing biomarker rates against recommended benchmarks as part of quality assurance activity.

258 Fibroepithelial Lesions Revisited

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Background: While the classic forms of fibroepithelial lesions (FEL) do not pose any diagnostic challenge, there are some that defy classification. The aim of this study was to review the morphologic spectrum of FEL in relation to outcome.

Design: We searched our files for phyllodes tumors (PT) and atypical fibroadenomas (aFA, size >5cm, moderate cellularity, mitoses >3 per HPF, leafy architecture, irregular border, ddx of FA vs. benign PT (BPT). All available slides were examined for border, architecture, stromal overgrowth, mitoses, necrosis, heterologous elements, and margin status. FEL were classified based on 2013 WHO book. Follow up (FU) was obtained from electronic medical records.

Results: We found 189 FEL in 177 patients (pt) at a median age of 43 y. There were 55aFA, 59BPT, 29 borderline (BoPT) and 46 malignant PT (MPT). Slides were available for 130 of them (45aFA, 42BPT, 20BoPT, 23MPT). FU was available for 143 pt (median 51m, range 3-264m); 3 died of disease (DOD, all MPT), 5 were alive with disease (AWD, 4 with metastatic MPT, 1 with recurrent BPT) and 135 had no evidence of disease (NED). Local recurrence rate was 14%. 11PT recurred with the same grade (8BPT, 2BoPT, 1MPT) and 3 as more aggressive (1BoPT-MPT, 2BPT-MPT); for 6 the original PT was unknown. Of 15 FEL with FU and positive margin 13 recurred and 2 had NED (p=<0.0001). The border of 13 PT with available slides that recurred was infiltrative in 9, pushing in 1 and indeterminate in 3 (p=0.0001). MI and ≥50% myxoid stroma were only predictive of recurrence in BPT (p=0.0278 and p=0.001). Among 31 aFA and BPT with pushing border that were enucleated none recurred (median 57m). Necrosis (31%) and heterologous elements (7%) were only seen in MPT; necrosis was predictive of metastatic event (p=0.002). Only MPT metastasized at 21% rate (at median 8m).

FEL	Median age (y,range)	Median size (cm,range)	Infiltrative border %	Mode of surgery % (lumpectomy, re-excision, mastectomy)	NED, AWD, DOD %
aFA	39 (17-67)	2.6 (1-10)	4	95,5,0	100,0,0
BPT	43 (13-82)	4 (0.7-14)	12	75,21,8	96,2,2*
BoPT	45 (12-60)	3.8 (1-14.5)	53	30,65,15	100,0,0
MPT	46 (15-70)	6.3 (1.9-24)	65	8,68,66	83,10,7

Table 1. Summary of findings; *recurredas MPT

Conclusions: In our study only positive margin and infiltrative border were statistically significant predictors of local recurrence, while necrosis was predictive of distant spread. Enucleation of aFA and BPT with pushing border appears to be a sufficient mode of treatment.

259 Mitotic Activity in Fibroepithelial Lesions

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Background: According to WHO Classification of Tumors of the Breast 2013 the usual mitotic activity per 10 HPF is none to rare in fibroadenomas (FA), low at <5 in benign phyllodes tumor (BPT), frequent at 5-9 in borderline phyllodes tumors (BoPT) and abundant at ³10 in malignant phyllodes tumors (MPT). The same authors also suggest that the mitotic count be related to the size of the field diameter (x40 objective and x10 eyepiece, 0.196mm^2). The aim of this study was to review the spectrum of mitotic activity in fibroepithelial lesions (FEL).

Design: After ethics approval we searched our records for excision specimens of PT with available slides. In addition we included 'atypical FA' (aFA, size >5cm, moderate cellularity, mitoses >3 per HPF, leafy architecture, irregular border, ddx of FA vs. BPT). Two mitotic counts were recorded: the highest mitotic score per 10 HPF from the pathology report and the highest mitotic score counted in a single most mitotically active 1.96 mm^2 area that was chosen after meticulous review of all available slides. Follow up (FU) was obtained from electronic medical records.

Results: We found 130 FEL with median of 6 slides per FEL (range 1-28). There were 45 aFA, 42 BPT, 20 BoPT and 23 MPT. The median tumor size was 3.7cm (range 0.7-24). When assessing MI in the most active area 17/45 (38%) of aFA had >3MF per 10HPF, 19/42 (45%) BPT had >5MF per 10HPF, 11/20 (55%) BorPT had >10MF per 10HPF, while only 5/23 (22%) MPT had <15 MF per 10HPF. The median FU was 55m (range 3-173m), during which 16 FEL recurred. MI was only predictive of recurrence for BPT, regardless of counting method (p=0.0278 using cut-offs 3 and 10, respectively).

FEL	n	MI BASED ON PATHOLOGY REPORT*			MI IN THE MOST ACTIVE AREA**		
		range	median	average	range	median	average
aFA	45	0-2	0	1	0-8	3	3
BPT	42	0-10	1	2	0-13	5	5
BoPT	20	1-10	5	5	3-20	11	11
MPT	23	1-25	10	13	7-48	21	25

Table 1. Summary of findings; MI – mitoticindex, *counted per 10HPF with no further specification of fielddiameter/area, ** counted in the single most mitotically active 1.96mm2 area.

Conclusions: Assessing MI in the single most mitotically active area results in significantly higher mitotic counts. The most mitotically active area, especially in aFA and BPT, is often present focally on a single slide. Mitotically active FA are fairly common. In our study mitotic index by itself is of limited value in predicting adverse outcome.

260 Impact of Heterogeneity for ki67 Index in Invasive Breast Carcinoma: A Study of 131 Consecutive Cases

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Background: In addition to conventional histopathological parameters, the assessment of proliferation is a major factor in treatment decision in breast carcinoma patients. Heterogeneity for ki67 is not well documented in the literature. The aim of this study was to assess weather ki67 heterogeneity in invasive breast carcinomas could have an impact over treatment decision.

Design: Immunohistochemistry for ki67 was evaluated in large tumor sections from resection specimens (mastectomies/lumpectomies) of 131 consecutive invasive breast carcinomas. Heterogeneity was defined as the presence of a low (less than 17%) and high (more than 35%) proliferative activity within the same tumor in the same histologic section. The rest of the cases were defined as homogenous. Clinical-pathological features of the cases were also analyzed.

Results: 107 (81.67%) of the cases were homogenous and 24 of 131 cases (18.32%) showed heterogeneity as defined above. Among these, 10 (41.6%) cases showed a gradient of increasing staining toward the tumor edge and 14 (58.4%) cases showed hot spots. In general, the proliferative activity varied from 1 to 90% in different areas of the tumor. A higher incidence of breast carcinoma was observed after menopause in both groups (83.33% in heterogeneous cases and 79.43% in homogeneous cases) (p=0.783). These groups were similar as far as the most frequent histological tumor types were concerned: NST type (95.83% vs. 56.07%)(p=0.0001). Tumor histological grade, T and N stage were similar in both groups. We noted a higher proportion of stage N3 cases in the heterogeneous tumor group (54.16% vs. 34.57%)(p=0.14). Prognostic markers analysis in the heterogeneous cases revealed 100% positivity for hormone receptors, (compared to 94.65%) and a much higher proportion of HER2 negative cases (87.5% vs. 73.83%)(p=0.19).

Conclusions: Ki67 heterogeneity can be seen frequently in breast carcinomas. Clinical-pathological features are similar in both groups analyzed, but with an increased prevalence of NST, more advanced clinical stages (N3) and more HER2 negative cases in the heterogeneous group. As ki67 value could have an impact on clinical decisions, it is mandatory to evaluate the whole specimen and not only the core biopsy specimen and to correlate it with mitotic count.

261 Mutivariate Clinic-Pathological Features Analysis for Detecting Unfavorable Independent Prognostic Parameters in Male Breast Cancer

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Background: Despite advances in prognostic factors in female breast cancer, data regarding the impact of different prognostic parameters including molecular markers is limited in male breast cancer. The objective of this study is to evaluate clinic-pathological parameters in correlation with overall survival (OS) to identify unfavorable independent prognostic parameters in male breast carcinoma.

Design: All male patients diagnosed with invasive breast cancer in three different international institutions between 2008-2013 were identified by searching laboratory databases. Parameters analyzed included age, clinical and pathological stage, histological tumor type and grade, molecular biomarkers, treatment and overall survival (5 year OS).

Results: 33 cases were identified. Mean age of patients was 64 (range: 45-81). Most of the cases were of NST histological type (69.69%), G2 histological grade (60.6%), T2 (63.63%), N2 (30.3%), M0 (93.93%) and Luminal A molecular subtype (64%). 25 patients received chemotherapy (CT), 9 of them received CT associated with hormone therapy (HT), 1 case only HT and 1 case- CT followed by radiotherapy (RT). Follow-up information was available in all patients ranging from 10 to 84 months. Median survival was 48 months. 21 patients are alive and free of disease (all of them received HT), 2 cases have metastatic disease and 11 patients are deceased. In univariate analysis a higher T status and the presence of axillary lymph node metastases was associated with worse median survival (p=0.014, respectively p=0.03) while in multivariate analysis we found that survival is independently influence by age (over 70 years unfavorable) (p=0.028, 95% CI=1.208-26.383). Among the deceased patients, the majority was treated only with CT while none of them received HT.

Conclusions: Older age is the only statistically significant unfavorable independent prognostic parameter in multivariate analysis in male breast carcinomas. While not statistically significant, all male breast cancer cases analyzed in this study had uniform expression of molecular markers (ER/PR positive-93.93%, HER2 negative-96.97%) and an excellent response to HT, which highlights its role in prognosis and survival.

262 Cancer Stem Cell and Epithelial-Mesenchymal Transition Markers Predict Worse Outcome in Triple Negative Breast Cancer

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Background: Triple negative (TN) breast cancers, defined by the absence of estrogen receptor, progesterone receptor and c-erbB2 (HER2) expression, have more aggressive clinical behavior, poorer prognosis, and lack targeted therapy options. Tumors with stem cell properties are able to self-renew, harbor a higher cancer recurrence rate and are more resistant to therapy, while epithelial-mesenchymal transition (EMT) predicts tumor progression, metastasis and drug resistance. We investigate immunohistochemical expression of cancer stem cell (CSC) and EMT markers in TN breast cancers.

Design: The study comprised 696 TN breast cancers (1994-2011) from the Department of Pathology, Singapore General Hospital. Antibodies to basal (CK14, 34βE12), EGFR, CD44 and EMT markers (E-cadherin and Snail2) were applied to tissue microarray sections, using the streptavidin-biotin method. CK14, 34βE12, EGFR, CD44 and Snail2 expression was defined as staining of 1% or more of tumor cells, with cytoplasmic decoration for CK14 and 34βE12, cytoplasmic membrane staining for EGFR, CD44 and E-cadherin, and nuclear reactivity for Snail2. Loss of membranous E-cadherin occurred when ≤70% of tumor cells expressed membranous staining. Disease free survival (DFS) and overall survival (OS) were calculated as time from diagnosis to recurrence or death respectively.

Results: Expression of CD44 and Snail2, loss of membranous E-cadherin was observed in 354 (51%), 215 (31%) and 274 (39%) tumors respectively. Histologic grade significantly associated with CD44 (p=0.007), Snail2 (p<0.001) positivity, loss of E-cadherin membranous expression (p=0.007). CK14, EGFR and 34βE12 confirmed 85% of cases to be basal-like, with similar correlation (CD44 positive, p<0.001; Snail2 positive, p=0.026; and E-cadherin loss, p=0.045). Positive Snail2 (p=0.007), loss of E-cadherin (p<0.001) were associated with syncytial growth pattern. CD44 positive tumors harbored pushing borders (p=0.001). Median follow up was 83 months, with a maximum of 236 months. A combinational phenotype (loss of E-cadherin, Snail2 and CD44 positivity) was observed in 57 (8%) tumors. Cases with this phenotype (p=0.013) and CD44 positive tumors (p=0.024) disclosed poorer OS. Multivariate analysis confirmed statistically significant association of the combinational phenotypewith unfavorable OS (HR 1.768, 95% CI 1,032-3.029, p=0.038).

Conclusions: We conclude that enrichment of CSC and EMT markers in TN breast cancers correlated with aggressiveness and basal-like phenotype, which may provide new insights for therapy.

263 Immunohistochemical Protein Expression of Chemokine Receptor Cxcr4 and Vegf Predicts Histological Grade and Poorer Outcome in Breast Phyllodes Tumors

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Background: Phyllodes tumors of the breast are uncommon fibroepithelial neoplasms which present challenges for grading and prognostic assessment. To date, there is no consensus regarding the role of biomarkers in these unusual tumors. Inhibition of CXCR4, a chemokine receptor, is believed to decrease vascular endothelial factor (VEGF) expression and angiogenesis in *vitro* and in *vivo*. The aim of this study was to evaluate protein expression of VEGF and CXCR4 in a series of phyllodes tumors and correlate the expression of these markers with clinicopathological parameters and outcomes.

Design: The cohort comprised 158 phyllodes tumors diagnosed at the Department of Pathology, Singapore General Hospital between 2005 to 2009, incorporating 107 (67.7%) benign, 39 (24.7%) borderline and 12 (7.6%)%) malignant tumors. Antibodies to CXCR4 and VEGF were applied to sections cut from tissue microarray blocks (2mm cores, 3 cores per case), using the streptavidin-biotin method. Immunoreactivity was assessed in stromal cells of tumors. Positive biomarker expression was defined as staining of mean percentage or more of stromal cells, with cytoplasmic membrane staining for CXCR4, and cytoplasmic decoration for VEGF. Disease free survival (DFS) and overall survival (OS) were defined as time from diagnosis to recurrence or death respectively, and correlated with protein immunohistochemical expression.

Results: Positive immunohistochemical expression of CXCR4 and VEGF was seen in 58 (36.7%) and 62 (39.2%) cases respectively. Stromal expression of CXCR4 and VEGF was significantly correlated with high histologic grade ($p=0.001$, $p<0.001$), permeative borders ($p=0.025$, $p=0.003$), stromal cell atypia ($p=0.021$, 0.001), and high mitotic score ($p<0.001$, $p<0.001$). CXCR4 expression was significantly associated with stromal hypercellularity ($p=0.036$), and VEGF was more expressed in larger tumors ($p=0.019$). At a maximun follow up duration of 75 months (mean and median 36 months), 11 patients developed recurrences, with three succumbing from phyllodes tumors. Patients whose tumors expressed CXCR4 and VEGF disclosed poorer OS ($p=0.015$, $p=0.034$ respectively).

Conclusions: Determination of CXCR4 and VEGF provides significant biological insight into breast phyllodes tumors. These markers may promote new therapeutic paradigms especially for the malignant group of phyllodes tumors.

264 Central Lab HER2 Testing By RT-PCR, IHC, & FISH for Quality Assurance (QA) in Locally HER2-Negative (HER2(-)), ER-Positive Invasive Breast Carcinoma (IBC) With Adjacent In Situ Carcinoma (IS)

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Background: Our HER2 QA program requires confirmatory testing w/ IHC & FISH at an outside central lab on all IBC samples w/ IS that are HER2-positive (HER2(+)) by central Oncotype DX (ODX) but are locally IHC/FISH HER2(-), equivocal (EQ), or unknown (UNK). The objective of this QA program is to further assess the quality of HER2 testing by RT-PCR & to identify rare intratumoral differences in HER2 expression between IBC & IS.

Design: From 12/1/10 - 8/26/14, RT-PCR HER2(+) IBC cases w/ IS which were locally HER2(-), EQ, or UNK were sent to a central lab (Vitro Molecular Labs; Miami, FL) for IHC/FISH testing using HER2 SP3 IVD assay & PathVysion, respectively. HER2 status was determined before 10/13 by the 2007 ASCO-CAP guidelines & after 10/13 by the 2013 revised ASCO-CAP HER2 guidelines. In cases of central IHC/FISH discordances (<2% of cases), HER2 status was recorded as (+) if at least 1 method was (+). RT-PCR for HER2 used ODX & the pre-defined HER2 cutoffs: (+) ^{3}11.5 units, EQ = 10.7 to 11.4 units, & (-) <10.7 units – each unit represents a 2-fold change in gene expression.

Results: 2,454 of 310,525 cases were RT-PCR HER2(+). 327 IBC cases w/ IS that were HER2(+) by RT-PCR & locally HER2(-), EQ, or UNK were sent for central lab IHC/FISH. 10 cases were excluded for inconclusive IHC/FISH due to preanalytic compromise. 317 IBC cases w/ IS were local HER2(-), EQ, or UNK in 212 (66.9%), 26 (8.2%), or 79 (24.9%) cases, respectively. 131 of 212 (61.8%) HER2(-) cases by local sites were HER2(+) by central IHC, FISH, & RT-PCR. 56 of 212 (26.4%) locally HER2(-) cases were found to have differences between IBC & IS by central IHC/FISH. 22 of 212 (10.4%) of locally HER2(-) cases were central IHC/FISH HER2(-).

	Local HER2(-) EQ or UNK N=317	**Local HER2(-) N=212**
RT-PCR concordant w/central IHC/FISH	**211 (66.6%)**	**131 (61.8%)**
RT-PCR+ vs central EQ	**7 (2.2%)**	**3 (1.4%)**
RT-PCR discordant w/central IHC/FISH	**99 (31.2%)**	**78 (36.8%)**
RT-PCR+ vs Central(-)	30 (9.5%)	22 (10.4%)
RT-PCR+ vs Central IBC(-)/IS+	69 (21.8%)	56 (26.4%)

Conclusions: RT-PCR testing has clinical utility in identifying pts who may be HER2(+) by central FISH/IHC & therefore candidates for HER2 targeted therapy. The QA program identifies rare cases where IS is HER2(+) & IBC is HER2(-).

265 Modified Magee Equations Predict Clinical Outcome for Breast Cancer Patients

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Background: The 21-gene assay (ODX) has been endorsed by ASCO and routinely used in guiding clinical decisions on chemotherapy for estrogen receptor (ER) positive, node negative breast cancer patients. Many investigators have tried to identify more effective and economical methods that have predictive powers similar to the 21-gene assay. In 2013, Klein et al reported that the Estimated Recurrence Scores (ERS) from three Magee equations derived by linear regression analysis demonstrated relatively high concordance with the RS of ODX. The current study aims to evaluate the clinical and prognostic utility of the three Magee equations.

Design: 560 patients with ER positive tumors, clinical follow-up and diagnosed between 1997 and 2012 in our institution were included. Modified ERS from all three equations, which used the Nottingham grade (3-9), modified H-score of ER and progesterone receptor (PR) (average staining intensity X percent positive tumor cells, 0-300), HER2 status (negative, equivocal, positive), tumor size (cm) and % labeling for Ki-67, were calculated from each case. A mean modified ERS of each case was used for statistical analysis.

Results: For this cohort, the mean age was 60 years, the mean tumor size was 2.17 cm, 71% were histologic grade 1 and 2, 63% were node negative. All patients were ER positive, 85% were PR positive, and 10% were HER2 positive. The Modified ESR was not associated with patient age, tumor size, or histological grade; but it was associated with nuclear grade and expression of ER, PR, HER2 and Ki-67. Although nodal status was not included in calculations in these equations, a high Modified ERS (>30) was strongly associated with positive node. Among the three components of Nottingham grading, only nuclear grade showed a significant association with Modified ERS. Only 4% of ER 71-100%, 2% of PR 71-100%, 8% of HER2 negative, and 5% of Ki-67<15% had Modified ERS >30. Also, Modified ERS was significantly associated with progression free survival in these 560 patients (p-value=0.0031) and in node negative subgroup of these patients (p value=0.0069), but not in node positive patients (p value=0.8697).

Conclusions: Modified ERS can effectively stratify ER positive patients into different prognostic subgroups. It may be used to guide clinical decision making for patients with ER positive tumors, in conjunction with other clinical-pathologic factors.

266 Pathological Features of DCIS of the Breast According To the Expression of Progesterone Receptor

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Background: Progesterone receptor (PR) is an estrogen receptor (ER)-regulated gene and has critical roles in both normal mammary and breast cancer development. Loss of ER expression in breast cancer is associated with older patients, larger tumors, higher grade tumors, and tumors over-expressing HER2. We previously showed that loss of PR expression predicts for higher Recurrence Scores (RS) for the 21-gene assay (ODX) and higher risk for bone metastasis. Recently, a 20% or less PR was proposed as a classifier to separate Luminal B from Luminal A subtype of breast cancer. Limited information is known for PR in ductal carcinoma in situ (DCIS). Here, we intend to study the relationship between the different levels of PR expression and various pathologic features in DCIS of the breast.

Design: 886 cases of primary DCIS of the breast from our institution and diagnosed between 1997 and 2013, had an exact percentage documented for ER and PR IHC scoring, were included in the study. The clinicopathological features, including patient age, tumor size, nuclear grade and expression of ER and PR, were collected for each case. The relationships between the different levels of PR expression (<1%, 1-20%, 21-50%, 51-70% and 71-100%) and these pathologic features were analyzed.

Results: Among the 886 cases of DCIS, the mean age was 85.5 years, mean tumor size was 2.4cm, 84.5% were positive for ER, and 76.2% were positive for PR. 24%, 14%, 13, 11%, and 38% of these DCIS had PR expression of <1%, 1-20%, 21-50%, 51-70% and 71-100%, respectively, similar in distribution as was seen in IDC (data not shown). Significant differences in patient age, nuclear grade and ER expression were observed only between the PR<1% and PR 1-20% subgroups, with less PR expression associating with younger patients, higher nuclear grade and lesion ER expression in DCIS; these difference was not observed between the PR 1-20% and PR 21-50% subgroups, the PR 21-50% and 51-70% subgroups, and the PR 51-70% and PR 71-100% subgroups. In ER positive tumors, the only significant difference noted between different PR subgroups was nuclear grade.

Conclusions: The significant pathological difference observed in between PR<1% and PR 1-20% suggests the cutoff of 1% for PR appears to be biologically important. Further studies are warranted to investigate the roles of PR in local/regional of DCIS of the breast.

267 Pathological Features of Invasive Ductal Carcinoma of the Breast According To Expression of Progesterone Receptor

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Background: Loss of PR expression in breast cancer is associated with older patients, larger tumors, higher grade tumors and tumors over-expressing HER2. We previously reported that loss of PR expression predicts higher Recurrence Scores (RS) of the 21-gene assay (Oncotype DX) and higher risk for bone metastasis. Recently, a 20% or less PR expression was proposed to separate Luminal B from Luminal A subtype of breast cancer. Here we intend to study the relationship between the different levels of PR expression and various pathologic features in invasive ductal carcinoma (IDC) of the breast.

Design: 3573 cases of primary IDC of the breast from our institution diagnosed between 1997 and 2013 and had an immunohistochemical (IHC) score for ER and PR in exact percentage were included in the study. The clinicopathological features (age, size, nodal status, lymphovascular invasion (LVI) status, histological grade (HG), and expression of ER, PR, HER2 and Ki-67) were collected for each case. The relationships between the levels of PR expression (<1%, 1-20%, 21-50%, 51-70% and 71-100%) and these pathologic features were analyzed.

Results: Among the 3573 cases, the mean age was 59.9 years, the mean tumor size was 2.05cm, 31% had nodal metastasis, 18% had LVI, 85%, 78% and 12.4% were positive for ER, PR and HER2 respectively. 53% showed Ki67 labeling equal or greater to 15%. Significant differences in LVI, HG and ER expression were observed between the PR<1% and PR 1-20% subgroups, and the PR 1-20% and PR 21-50% subgroups, with higher incidence of LVI, higher HG and lower ER expression associated with the subgroups having lower PR expression. These differences were not observed between the subgroups of PR 21-50% and 51-70%, or PR 51-70% and PR 71-100%. In ER positive tumors, significant difference was noted for all pathologic features studied (tumor size, nodal status, LVI status, histological grade, and expression HER2 and Ki67) except for patient age. Similar differences were also identified for these subgroups in HER2 negative tumors, but not in HER2 positive tumors.

Conclusions: The difference observed in between PR<1% and PR 1-20% and between PR1-20% and PR 21-50% suggests that the cut offs of 1% and 20% for PR may be biologically important for IDC. The different levels of PR expression may play critical roles in ER positive and HER2 negative breast cancer.

268 Prognostic Significance and Independent Predictive Value of CBP (CREB-Binding Protein) in Breast Cancer (BC)

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Background: *CREBBP* encodes CREB binding protein (CBP) which plays a critical role in embryonic growth and development and regulation of gene expression. It has been unclear whether CBP functions as a tumor suppressor or oncogene in cancer progression. In this study, we measured the prognostic significance of CBP expression in a large cohort of invasive BC patients.

Design: Formalin-fixed, paraffin-embedded tissue sections from 88 cases of invasive BC [68 ductal carcinomas (IDC) and 20 lobular carcinomas (ILC)] were immunostained by a manual method (DAKO EnVision+ Dual Link System-HRP) using rabbit polyclonal CBP (Santa Cruz Biotech, Santa Cruz, CA). Nuclear and/or cytoplasmic immunoreactivity was scored based on intensity and percentage of positive tumor cells in both the tumor and adjacent benign epithelium (when present) in each case. Results were correlated with morphologic and prognostic variables.

Results: CBP immunoreactivitywas noted as nuclear [42/88 (48%) tumors] and cytoplasmic [43/88 (49%) tumors]. Nuclear CBP overexpression correlated with tumor type [14/20 (70%) ILC vs 28/68 (41%) IDC, p=0.023], and overall with low tumor grade (p=0.026), ER positive status (p=0.017), PR positive status (p=0.003), HER2 negative status (p=0.023), and longer survival on Cox univariate analysis (p=0.02). Cytoplasmic CBP overexpression correlated overall with advanced tumor stage (p=0.018); and within the ILC subgroup correlated with advanced stage (p=0.019), lymph node positive status (p=0.05), disease recurrence (p=0.028), and showed a trend toward association with shortened survival on Cox univariate analysis (p=0.07). On multivariate analysis, disease recurrence (p<0.0001), positive LN status (p=0.012) and absence of nuclear CBP overexpression (p=0.035) were independent predictors of shortened survival.

Conclusions: CBP appears to function as a tumor suppressor in BC as loss of nuclear CBP expression is an independent predictor of shortened survival for the disease. Further study of the biologic and clinical significance of CBP in BC appears warranted.

269 4E-T Is Associated With Invasiveness in Breast Carcinomas: A New Target Associated With N-Cadherin Expression

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Background: Tumor invasiveness and metastasis are associated with prognosis and epithelial mesenchymal transition (EMT) plays a central role. Deregulated protein synthesis is an important and targetable feature of cancer progression. The translation initiation factor eIF4E has been associated with invasiveness and poor prognosis in breast cancer but little is known about the closely associated eIF4E transporter and P-body component 4E-T.

Design: Eighty breast carcinomas were analyzed by immunohistochemistry and western blot for the expression of 4E-T, pMAPK, mTor, 4E-BP1, p4E-BP1, eIF4E, peIF4E, and the EMT factors N-cadherin, Snail, Slug and vimentin. Breast carcinoma cell lines (MDA-MB 231, MBA-MB 468) were infected with retrovirus to overexpress 4E-T and lentivirus for shRNAi-mediated knockdown of 4E-T (sh4E-T). Cells were subjected to migration and invasion assays in vitro. In mice, both orthotopic and tail injections of MDA-MB-231 cells were performed and tumors were removed when reaching a size of 5 mm.

Results: In human breast tumors the levels of 4E-T correlated with N-cadherin expression ($p < 0.0018$) and both correlated to size ($p < 0.01$). In vitro analysis showed an increase of migration and invasiveness in cells overexpressing 4E-T ($p < 0.01$). In mice there was a significant increase in local relapses in the orthotopic model (100% relapsed in 4E-T vs 33% both in sh4E-T and control), and also in the number and size of the lung metastasis in the intravascular injection model ($p = 0,018$), where metastatic load was 2.87 times higher in 4E-T overexpressing tumors versus sh4E-T, and 1.64 times higher than control.

Conclusions: We describe a novel prognostic role of 4E-T, which is associated with N-cadherin and with local invasion and metastasis. The pathway through which 4E-T upregulates N-cadherin and promotes invasion is under intense investigation. These results open a new avenue of research to identify targets associated with relapses and metastases.

270 The Immune Microenvironment of Breast Ductal Carcinoma In Situ: A Pilot Study

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Background: The host immune response plays a role in breast cancer progression and response to therapy. In invasive breast cancers, higher numbers of cytotoxic T cells and B cells, and fewer FoxP3+ regulatory T cells, are associated with improved survival. We previously reported differential patterns of tumor infiltrating lymphocytes (TIL) across subtypes of matched primary and metastatic breast carcinomas. The immune milieu of breast ductal carcinoma *in situ* (DCIS) has not been studied. Here we profile TIL phenotype and PDL1 expression (a component of a major immune checkpoint pathway) in 27 cases of DCIS with characterization of estrogen receptor (ER), progesterone receptor (PR) and HER-2 expression.

Design: Tissue microarrays (TMAs) containing 27 cases of primary DCIS were stained for PDL1, CD3, CD4, CD8, FoxP3 (regulatory T cells), and CD20 to characterize TILs. TMAs were labeled for ER, PR and HER-2 to classify tumors as Luminal A (ER+/PR+/HER-2-), Luminal B (ER+/PR+/HER-2+), HER-2+ (ER-/PR-/HER-2+) and triple negative (TNBC; ER-/PR-/HER-2-). Average TIL per high power field (HPF) was counted. Tumor and immune cell PDL1 expression were scored with any membranous labeling >5% considered positive. Clinicopathologic data were collected.

Results: The average age of patients with DCIS was 41 years, with 52% <40 (young) and 48% ≥40 (older). 16 cases (59%) were Luminal A, 4 (15%) Luminal B, 3 (11%) HER-2, 3 (11%) TNBC; 1 was not evaluable. DCIS in young women (<40) and ER-negative DCIS contained higher numbers of all TIL subsets. Younger women were more likely to have DCIS with a CD8/FoxP3 ratio >4 than older women. Fewer cases of ER-negative than ER-positive DCIS had a CD8/FoxP3 ratio >4. No DCIS expressed PDL1, but the majority of cases contained PDL1-positive TILs. 3 patients developed local recurrences. DCIS that recurred contained higher numbers of all types of TIL and a lower CD8/FoxP3 ratio than DCIS that did not recur.

Conclusions: This pilot analysis is the first study to evaluate TIL phenotype and PDL1 expression in breast DCIS. We demonstrate that differential TIL patterns are seen in ER-negative relative to ER positive DCIS, as well as in DCIS in younger relative to older women. We also show that, while the majority of DCIS contain PDL1-positive TIL, none of the DCIS cells expressed PDL1. Our findings suggest that further characterization of the DCIS immune microenvironment may yield targets for immune-based therapy and prevention.

271 Effects of Intermediate Oncotype DX Score on Oncologist Decision-Making

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Background: The NCCN Guidelines suggest considering adjuvant chemotherapy (cx) in patients with early stage ER positive invasive breast cancer >0.5 cm and negative lymph node status. The Oncotype DX assay is a 21-gene assay that has been used in these patients to predict the likelihood of recurrence as well as the benefit of adjuvant cx. Patients with a high recurrence score (RS) (>30) are more likely to benefit from the addition of adjuvant cx agents, while those with a low RS (<18) will have minimal to no benefit and may forgo the additional cx. Patients with an intermediate RS (18-30) have mixed results with the addition of cx. The goal of this study is to determine how the intermediate RS correlates with treatment recommendations.

Design: 199 cases with available Oncotype DX results from 2006-2011 were identified. Pathology reports were reviewed for patient tumor characteristics, including grade, size, ER, PR, and Her2neu status. Electronic medical records were reviewed for age, menopausal status, treating oncologist, and treatment recommendations. Statistical analysis was performed. A P value <0.05 was considered statistically significant.

Results: Of the 199 cases with available Oncotype DX results, 89 (45%) had intermediate RS. All cases were ER positive, Her2 negative, and T1b-T2. Of the 89 intermediate cases, 42 (47%) were treated with adjuvant cx, while 47 (53%) were treated with hormonal therapy alone. The RS did not correlate with tumor grade, size, mitotic activity, or ER positivity. There was an inverse correlation between RS and PR positivity (p=0.03). Patients treated with cx were younger (58 vs. 63, p=0.01) and had higher RS (24 vs. 22, p=0.003). With each incremental increase in RS, the odds of choosing cx

increased by 23.5% (OR=1.235, p=0.010). With each additional year of age, the odds of choosing cx decreased by 5.3% (OR=0.947, p=0.058). The decision to treat with adjuvant cx did not correlate with menopausal status, oncologist, size or grade of the tumor. Of note, 3 of 3 patients with N1mi nodal status were treated with cx (RS 18-24), and the 1 patient with N0(i+) nodal status was treated with hormonal therapy alone.
Conclusions: Patients with intermediate Oncotype DX RS are equally likely to receive cx as hormonal therapy. Younger patient age and higher numerical value of the RS show a positive correlation with the decision to use cx. Menopausal status, tumor size and tumor grade did not influence treatment decisions in this group of patients.

272 Survivin Is a Poor Prognostic Factor in Triple-Negative Breast Cancer

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Background: Survivin is a member of the inhibitor of apoptosis gene family and has dual roles in promoting cell proliferation and preventing apoptosis. It is one of the 16 genes tested in Oncotype DX, an RT-PCR assay used to predict additional benefit from chemotherapy in estrogen receptor positive, lymph node negative breast cancer patients. We set out to investigate the prognostic significance of Survivin in triple-negative breast cancer, the most aggressive subtype currently lacking targeted therapies.
Design: With Queen's University Research Ethics Board approval, a cohort of 348 breast cancer patients with 5-12 years of follow-up was assembled. A rich clinical database was created and tissue microarray was constructed. ER-/PR-/HER2- tumours were defined as triple-negative. The immunohistochemistry-based subtype definitions were luminal (ER+ or PR+/HER2-), luminal/HER2+ (ER+ or PR+/HER2+), HER2+ (ER-/PR-/HER2+) and triple negative, including the basal (ER-/PR-/HER2-/CK5+ or EGFR+) and non-basal (ER-/PR-/HER2-/CK5-/EGFR-) subgroups. Proliferation index was assessed using Ki-67 and Survivin stains. Nuclear staining for Survivin was scored based on the percentage of positive cells as follows: 0, 1-10%, 10-50% and >50%.
Results: There were 53 triple-negative (15%) patients (including 43 basal, 8 non-basal and 2 unclassifiable cases), 213 luminal (61%), 21 luminal/HER2+ (6%), 24 HER2+ (7%) and 37 (11%) unclassifiable breast cancers. Survivin was not differentially expressed in the luminal subtypes. Overall survival was lower in triple-negative cases versus the rest of the cohort. Unadjusted univariate analysis showed that high Survivin expression (>50% positive cells) versus low (<50% positive cells) Survivin expression predicted for lower overall survival (p=0.004). Survivin expression in >10% cells was associated with lower recurrence-free survival (p=0.049).
Conclusions: Increased expression of Survivin at the protein level merits further study as a possible prognostic factor in triple-negative breast cancer patients.

273 Reflex Estrogen Receptor (ER) and Progesterone Receptor (PR) Analysis of Breast Ductal Carcinoma In Situ in Core Needle Biopsy Specimens Significantly Increases Health Care Costs

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Background: ER and PR have traditionally been assessed on whole sections of excisional specimens (EXS) for breast cancer to determine eligibility for hormone therapy. Clinicians now ask that ER and PR be reflexively assessed on all core needle biopsy specimens (CNBX) showing ductal carcinoma in situ (DCIS), despite the fact that these results do not impact the next step in therapy; namely, surgical excision. However, a subset of EXS performed for DCIS diagnosed on CNBX will harbor IDC which would need to be retested for ER/PR. Moreoever, because ER and PR labeling is often heterogeneous, many recommended that negative results for these markers on small CNBX be repeated on EXS. We have previously demonstrated that reflex ER/PRHer2 testing of invasive mammary carcinomas (IMC) on CNBX unnecessarily increases costs due to the need to repeat negative results in EXS (Mod Pathol 2014;27(S2): 86A). We now examine the added cost associated with reflex ER/PR testing of DCIS on CNBX.
Design: We reviewed all cases of CNBX showing pure DCIS which also had a resulting EXS at our institution over a period of 2 years. Cases in which ER or PR were inadvertently not ordered on CNBX were excluded. EXS lesions which could not be definitively matched with the DCIS on CNBX (e.g. some EXS with multiple tumors) were excluded. The number of cases in which the EXS harbored IMC, as well as the number of cases in which ER/PR was negative on the CNBX, was assessed. The cost of each test was calculated as both the technical and pro-fee bill combined.
Results: A total of 63 cases of DCIS meeting the study criteria were identified. 6 (10%) only had benign pathology on EXS, 44 (70%) had pure DCIS, 4 (6%) had DCIS with microinvasive carcinoma (MI), and 9 (14%) had DCIS with IMC. The 13 cases with MI or IMC (20% overall) required repeat ER and PR testing of the invasive component in the EXS. Of the 44 cases with pure DCIS, 4 were negative for both ER and PR on CNBX which also merited repeating testing on EXS. The total increased cost of repeat testing on these cases was $10,656 dollars.
Conclusions: Reflex ER/PR testing of DCIS on CNBX unnecessarily increases health care costs. Extrapolating our cost of $201 per DCIS diagnosis on CNBX to 50,000 new cases of DCIS in the United States each year, reflex ER/PR testing would increase costs by over $10 million dollars. As with IMC, we recommend that ER and PR not be reflexively ordered on CNBX specimens containing DCIS, but instead be routinely performed on EXS.

274 High Ezrin Expression Is Linked To Poor Prognosis and Drug Resistance in Estrogen Receptor-Positive Breast Cancer

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Background: Over-expression of the metastasis-associated protein ezrin has been identified in many invasive cancer types including breast, but has not yet been associated with a specific breast cancer (BC) subtype. Furthermore, recent studies have indicated a link between ezrin and drug resistance. However, very few studies have been conducted for BC, or have assessed whether ezrin is associated with treatment response.
Design: Using an open access gene expression database (KM Plotter), we looked at ezrin expression with overall survival (OS) and distant metastasis-free survival (DMFS) in estrogen receptor positive (ER+) and negative (ER-) BC patients. To understand the role of ezrin in drug resistance, we over-expressed or silenced ezrin expression in MCF-7 and ZR-75-1 BC cells, which have low and high ezrin levels, respectively and show differential intrinsic sensitivity to various chemotherapeutic agents. Cytotoxicity assays were performed on cells treated with doxorubicin (Dox). Western blotting was performed on whole cell lysates to assess changes in the PI3K and NF-kB survival signaling pathways which have previously been linked to ezrin.
Results: Based on the KM Plotter analysis, high ezrin expression was associated with a lower OS and DMFS in a cohort of ER+, but not ER-, BC patients. In vitro assessment of high vs low ezrin expression revealed that over-expression of ezrin in MCF-7 cells which are sensitive to Dox, increased resistance to the cytotoxic drug, while ezrin-depletion in ZR-75-1 cells which exhibit intrinsic resistance to Dox, resulted in increased sensitivity to Dox treatment. Biochemical analysis demonstrated that over-expression of ezrin increased phosphorylation of Akt, p65 NF-kB, and expression of its downstream target, survivin. Furthermore, ezrin-depletion reduced p65 NF-kB phosphorylation and survivin expression, but did not alter Akt phosphorylation.
Conclusions: High ezrin expression may be a prognostic marker for poor outcome in ER+ BC patients. Furthermore, high ezrin expression is associated with increased drug resistance and NF-kB/survivin signaling, which may provide important survival signals for the ezrin-mediated drug resistance phenotype. Analysis of our locally accrued breast tumour cohort (n=348) is in progress to validate the ezrin pathway as a potential predictive marker of treatment response.

275 Next-Generation Sequencing Refines Natural History of Breast Carcinomas

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Background: Molecular mechanisms mediating the disease progression of ductal carcinoma *in situ* (DCIS) to invasive ductal carcinoma (IDC) of the breast remain unknown. Our aim was to decipher somatic variants and copy number alteration(s) which could favor progression from DCIS to IDC and axillary lymph node metastasis (N+).
Design: Two cases were analyzed (Patient 1: DCIS in 2004; relapse in 2010: IDC associated with DCIS; Patient 2: IDC associated with DCIS and N+). All components and their peritumoral normal tissue were retrospectively microdissected on frozen tissue sections for DNA extraction. Paired-end whole-exome sequencing was performed (average depth of coverage 70X; uniquely mapped reads). Single-nucleotide variants (SNVs) were identified with SAMTools and GATK. After evaluation of their putative functional impact (PolyPhen-2 and GERP scores), SNVs were validated with Sanger sequencing. Copy-number genomic profiles were determined from whole-exome datas (The Control-FREEC algorithm). To determine if SNVs identified in only one component could be detected in the others, targeted ultra-deep sequencing was performed.
Results: SNVs in *PIK3CA* and *CBFB* were identified in all tumor components of both patients. For patient 2, SNVs in genes such as *TP53*, *EIF4A1*, *ABCD3* and *GATA3* were identified in all tumor components. Ultra-deep sequencing revealed that all 10 SNVs initially observed in only one tumor component only, were present in all tumor components. Strikingly, two SNVs in *CDKL1* and in *CNTNAP2* genes were identified at much lower allelic frequencies in IDC compared to that of DCIS and N+. Furthermore, for patient 2, somatic copy number alteration profiles revealed a X chromosome subclonal loss in DCIS and a clonal loss IDC, verified by FISH but was not present in the axillary lymph node metastasis.
Conclusions: Our study showed that despite X chromosome loss visible in 100% of the IDC cells, the N+ cells harbored no X chromosome loss, suggesting that metastatic cells could derive from subclones of the DCIS component and not from IDC in this patient. The mutational profile was also consistent with this observation. These whole-exome sequencing data refined the mutation repertoire of DCIS and showed that DCIS, IDC and N+ from a same patient harbored identical but also private somatic genomic alterations and highlighted inter-tumoral as well as intra-tumoral heterogeneity.

276 Utility of Immunohistochemical Expression of C-KIT (CD117) in Categorization of Benign Versus Atypical/ Malignant Breast Lesions and Comparison With Other Differentiation Markers

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Background: C-kit (CD117) is a transmembrane tyrosine kinase that is thought to be lost during malignant transformation of the mammary epithelial cells. In this study we compared the expression of C-kit in benign and malignant lesions of the breast with the traditional known differentiation markers (high molecular keratins; CK903 and CK5/6) in assessment of difficult breast lesions such as usual duct hyperplasia (UDH) versus atypical ductal hyperplasia (ADH), in-situ and invasive carcinomas.

Design: Immunohistochemistry for CD117, CK903 and CK5/6 was performed on 166 cases of breast lesions including invasive (66) and in-situ (54) mammary carcinoma, adenoid cystic carcinoma (ACA) (3), metaplastic carcinoma (MCA) (1), flat epithelial atypia (7), atypical ductal hyperplasia (5), fibroadenomas (5), fibrocystic disease (6), sclerosing adenosis (4), microglandular adenosis (3), radial scars (4) and UDH (8). The adjacent normal duct epithelium was observed as normal control.

Results: CD117 expression was absent in 98.5% invasive mammary carcinomas (both ductal and lobular), 100% of in-situ carcinomas and ADH. CD117 expression was noted in 100% cases of ACA and MCA. CK903 was lost in 79% invasive carcinomas (excluding invasive lobular carcinoma), 80% in-situ carcinomas and 40% cases of ADH. CK5/6 was lost in 87% invasive carcinomas, 98% in-situ carcinomas and 40% cases of ADH. There was no cross reactivity of CD117 with myoepithelial cells. CK903 and CK5/6 were expressed in the myoepithelial in all cases (100%). 20% of the total malignant cases which could not be clearly categorized as neoplastic by CK903, could be clearly categorized as neoplastic by demonstrating loss of C-kit (statistically significant, $p<0.001$). A heterogenous CD117 expression was seen in all (100%) benign proliferative lesions. CD117 expression was observed in the stromal myofibroblasts and mast cells.

Conclusions: Expression of CD117 was indicative of benign breast lesions while loss of expression was seen in situ and invasive carcinomas and atypical proliferative lesions. C-kit was a more reliable immunomarker than either CK903 or CK5/6 which consistently showed co-expression in myoepithelial cells in in-situ carcinomas and atypical epithelial hyperplasia. These results also suggest that loss of C-kit may be one of the key molecular events in mammary carcinogenesis. More studies are required to validate the role of C-kit as a diagnostic marker in breast neoplasms.

277 Encapsulated Papillary Carcinoma With Metaplasia

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Background: Benign papillary lesions infrequently undergo squamous metaplasia, usually secondary to infarction. We sought to investigate the frequency of divergent, metaplastic differentiation in patients with encapsulated papillary carcinoma, the malignant counterpart and rare special subtype of breast carcinoma, which usually has a ductal epithelial phenotype.

Design: 39 cases of encapsulated papillary carcinoma, with or without an associated frankly invasive component, were identified from institutional (N=27; 5/06-9/14) and consultation (N=12; 7/11-9/14) files. Clinicopathologic features were assessed by slide and chart review.

Results: Patients had a mean age of 64 (range 35-82). 16 of 39 (41%) had associated frank invasion (3 micro- and 13 macro-invasive). Invasive carcinoma was cytologically similar to the associated encapsulated papillary carcinoma in all cases. Nodal status was as follows: 16 (41%) N0, 2 (5.1%) N1mi, 6 (15.4%) not done and 15 (38.5%) unknown. Encapsulated papillary carcinomas had mean size of 1.7 cm (range 0.5-4.3 cm); had the following grade: 15 (38.5%) low, 18 (46.2%) intermediate and 6 (15.4%) high; ER/PR expression: 26 (66.7%) positive, 4 (10.3%) negative and 9 (23.1%) unknown and HER-2/*neu* status: 21 (53.8%) negative, 1 (2.6%) positive, 8 (20.5%) not done and 9 (23.1%) unknown. When frank invasion was present, mean size of the invasive component was 0.8 cm (range 0.05-3.2 cm).

One or more myoepithelial markers were perfomed in 27 of 39 (69.2%) cases, 6 with and 22 without frank invasion, with the following staining patterns in the encapsulated papillary carcinoma component: 15 (55.6%) complete lack; 8 (29.6%) focal, patchy, predominantly peripheral; and 4 (14.8%) diffuse peripheral and papillae staining.

Divergent differentiation was present in 2 (5.1%) cases, one with and one without an associated invasive component. Both had squamous morphology, supported by positive staining with p63 (see below). Of note, another case had anaplastic features; this case had associated invasive ductal carcinoma and was HER-2/*neu* positive.

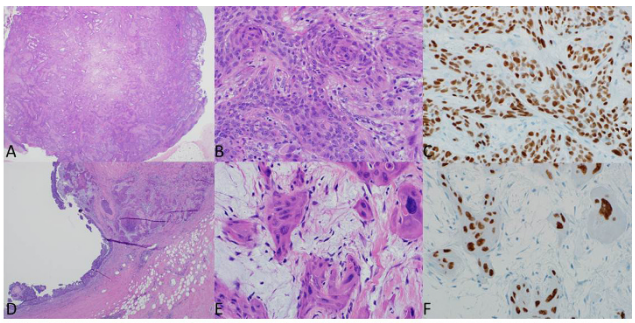

Figure 1. Encapsulated papillary carcinoma with squamous differentiation (A) (H&E, 2X) (B) (H&E, 40X) and positive p63 staining (C) (IHC, 40X) and encapsulated papillary carcinoma with associated frank invasion, both with squamous differentiation (D) (H&E, 2X) (E) (H&E, 40X) and positive p63 staining (IHC, 40X).

Conclusions: Metaplastic differentiation in encapsulated papillary carcinoma is rare and in our cohort was limited to squamous phenotype.

278 The Basal-Like Subset of Luminal-B Breast Cancers

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Background: About 20% of breast cancers are currently classified as luminal-B (LBBC). However, gene expression analyses and outcome studies have shown heterogeneity within the subtype. Furthermore, although 50-75% basal-like tumors are triple negative immunophenotype, the other 25-50% is distributed in the HER2 and luminal subtypes. This study was designed to evaluate basal-like markers in LBBC.

Design: Formalin fixed paraffin embedded TMAs were constructed from 180 LBBC (two 1 mm cores per tumor). The cases included were ER+ and/or PR+, HER2+ (or HER2 negative with Ki67 ≥ 14%). The TMAs were immunostained using EGFR (clone 3C6; Ventana, Tucson AZ), CK5/6 and CK14 (both Cell Marque, Rocklin CA) antibodies. For each case and each biomarker, any extent and intensity of cytoplasmic/membranous immunoreactivity observed in invasive cancer in ≥ 1 of the 2 cores was scored as positive. Absence of staining in invasive cancer in both cores was recorded as negative. In-situ carcinoma was not evaluated. Using $P<0.05$ as significant, EGFR, CK5/6 and CK14 expression in invasive tumors was correlated with age and tumor size, modified Bloom-Richardson grade (MBR), lymph node status, ER%, PR%, Ki67%, p53%, HER2 by immunohistochemistry and by FISH.

Results: The patients ranged from 27 to 91 years in age (median 58 yrs) at diagnosis and the tumors ranged from 0.4 to 10 cm (median 2 cm). 42 (23.3%) cancers expressed ≥ 1 of the 3 basal-like markers (EGFR, CK5/6, CK14) with 11 expressing all 3. Significant differences in MBR, ER%, PR% and Ki67% were observed when tumors positive for all 3 markers were compared to the other 169 tumors. Tumors positive for all 3 markers showed higher MBR and Ki67% and lower ER% and PR% ($P<0.05$).

Conclusions: -About 6% of cancers currently classified as LBBC express all 3 basal-like markers (EGFR, CK5/6, CK14).

-The higher MBR and Ki67% and lower ER% and PR% characteristic of this subset would indicate a poor prognosis.

-Correlation with outcome will determine if these markers would be useful clinically for stratification of LBBC.

279 LCIS-Associated Myoepithelial Cells: Distribution and Immunophenotype

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Background: In 1980, Bussolati elegantly characterized the relationship of lobular carcinoma in situ (LCIS) and myoepithelial cells (MECs) using anti-actin immunohistochemistry and electron microscopy. The patterns they described included: MECs flattened to the basement membrane (BM, pattern 1), perpendicular to it (pattern 2), or intermingled with LCIS (pattern 3). Recent molecular and immunohistochemical studies have demonstrated that ductal carcinoma in situ-associated myoepithelial cells are different from MECs in normal breast. The molecular and phenotypic characterization of LCIS-associated MECs remains largely unclear. In this study we sought to revisit the distribution and characterize the phenotypic features of LCIS-associated MECs.

Design: With IRB approval, 20 breast resection cases with LCIS were identified from pathology archives; most had accompanying invasive lobular (13) or ductal (3) carcinoma. Representative blocks were stained for E-Cadherin/smooth muscle myosin heavy chain (SMMHC) dual stain, CK7/p63 dual stain, SMMHC alone, smooth muscle actin (SMA), and calponin. In each case, the intensity and distribution of staining in LCIS-associated MECs were compared to the MECs surrounding normal breast tissue on the same slide.

Results: In 70% of the cases, LCIS-associated MECs demonstrated decreased staining intensity for one or more myoepithelial markers when compared with normal MECs. Reduced staining was observed in 55% of LCIS for calponin, 45% for SMMHC, 25% for SMA and 5% for p63. In 10% of the cases, LCIS-associated MECs showed increased expression for one or more myoepithelial markers, usually calponin. Multiple architectural patterns of LCIS and MEC were apparent in the same section for most of the cases. All cases had pattern 1 (flat); pattern 2 (perpendicular) was observed in 75%, and pattern 3 (intermingled) was seen focally in 20% of the cases. Foci with p63+ nuclei one cell layer above the BM were seen in 90%; in 6 cases cytoplasmic MEC staining encircled cells in the outermost LCIS layer. In one involved nipple duct, MECs bridged between the BM and residual ductal epithelium.

Conclusions: LCIS-associated MECs display immunophenotypic alterations as compared to MECs associated with normal breast epithelium. Given diminished staining, our study favors application of several MEC stains, including p63, for best sensitivity. The pathologic significance of the varied MECs distribution and immunophenotype remains to be determined.

280 hTERT Gene Expression and DNA Methylation in Breast Cancer Revisited By Realtime RT-PCR and Methylation Specific PCR

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Background: A study conducted on tumor cell lines by a Johns Hopkins group on DNA methylation in two CpG island rich regions of the *hTERT* gene promoter showed little or no methylation in the region around the transcription start site, but hypermethylation in the -600 bp region. The group concluded that such unmethylated status is required for *hTERT* gene expression despite dense methylation elsewhere in the promoter. Given the potential clinical significance as a molecular biomarker, we explored if such findings are duplicable on surgically resected breast cancers. We also examined the possible correlation of DNA methylation with *hTERT* gene expression.

Design: DNA and total RNA from tumors and normal breast of resected specimens were extracted. Quasi-quantitative methylation specific PCR was conducted on bisulfite

treated DNA after determining linear range of amplification on titrated DNA samples. The agarose gel images were quantitated with TotalLab Quant software. Tissue microdissection was also performed on formalin fixed paraffin embedded (FFPE) tissue blocks. Realtime RT-PCR was performed using *GAPDH* gene as the endogenous control and the *hTERT* gene expression level was calculated as percentage of the MCF7 breast cancer cell line.
Results: Unmethylated CpG islands around transcription site were seen in both tumors and normal breast from 19 out of 26 cases. In contrast, a generalized hypermethylation about 1.5-2 folds was observed in tumors relative to normal breast in the -600 bp region. The hypermethylation was also confirmed on additional 24 breast cancer cases. The results were reproducible on both fresh and FFPE tissue. Realtime RT-PCR on the 50 cases showed no *hTERT* gene expression in 15 cases; expression in 22 cases (from 0.2% to 370% MCF7); and RNA degradation in 13 cases. Further data culling showed that the hypermethylation appears to promote *hTERT gene* expression in a subset of invasive ductal carcinoma.
Conclusions: Our findings help clarify literature by demonstrating that lack of DNA methylation around the transcription site can be seen in both tumors and normal breast, thus such unmethylated status is not required for the *hTERT* gene expression. In addition, the evident tumor DNA hypermethylation in the -600bp region can be further explored as a biomarker to aid in diagnostics of difficult breast cancer cases. The results also contribute to the existing pool of evidence that associate DNA methylation patterns with *hTERT* gene expression in breast cancers.

281 Total (TIL-t) and CD8+ Tumor-Infiltrating Lymphocytes (TIL-CD8) Predict the Extent of Luminal/HER2- Breast Cancer (BC-Lum) Pathological Response To Neoadjuvant Cytotoxic Therapy (NACT)

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Background: Except the degree of tumor cell proliferation, no marker predictive of the BC-Lum response to NACT has been validated to date. TIL amount was recently demonstrated to predict NACT effect in the HER2+ and triple-negative BC, however the importance of pre-NACT TIL for the response of BC-Lum to cytotoxics has not been fully explored. We investigated whether the baseline TIL amount in BC-Lum could be related to the extent of response to NACT.
Design: TIL amount was evaluated in pre-NACT tumor biopsies of 68 BC-Lum patients (pts). NACT consisted of taxane (T) plus anthracycline (A) (41 pts), T only (3 pts) or A only (14 pts). Both TIL types were evaluated on 10 consecutive high-power fields (field diameter 0.625 mm) : TIL-t on HES-stained sections and TIL-CD8 by immunohistochemistry. TIL-t amount was defined as % of tumor stroma occupied by TIL-t. TIL-CD8 amount was the number obtained by counting the CD8+ TIL. TIL-t and TIL-CD8 were correlated to patient age, pre-NACT tumor size, histotype, grade, Ki67 index, luminal subtype, lymph node status, response to NACT and post-NACT residual tumor (RT) size.
Results: The pathologic complete response (pCR) rate was 10% (Sataloff's classification, PMID:7874340). It was significantly higher among the pts with TIL-t > 15% (29%) than among those with TIL-t ≤ 15% (3%), p=0.01. Similarly, the pCR rate of the pts with TIL-CD8 > 218 was significantly higher than the one of the pts with TIL ≤ 218 (26% vs 0%, resp., p=0.006). In addition, significantly more pts with >25% TIL-t had a small post-NACT RT (≤ 10 mm) than the pts with TIL-t ≤ 25% (77% vs. 11%, resp., p=0.0000028). The baseline TIL-CD8 > 218 also predicted for a post-NACT RT ≤ 10 mm (53% among the pts with TIL-CD8 > 218, 6% among those with TIL-CD8 ≤ 218, p<0.00024). No other parameter could predict the pCR rate and the RT size in these pts. The only correlation between a clinico-pathological parameter and TIL was significantly higher TIL-t in ductal than in lobular BC-Lum (p=0.027).
Conclusions: The baseline TIL amount was highly predictive of pCR rate and residual tumor size of BC-Lum treated by NACT. TIL-t and TIL-CD8 appeared as simple and powerful biomarkers able to indicate which BC-Lum pts will benefit from NACT. Our method developed for TIL-t and TIL-CD8 assessment in pre-NACT BC tissue warrants further investigation on larger cohorts.

282 GATA-3 Expression Is Not Associated With Complete Pathological Response in Triple Negative Breast Patients Treated With Neoadjuvant Chemotherapy

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Background: Triple negative breast cancer (TNBC) is defined by the lack of estrogen, progesterone, and HER-2 expression. Even with optimal management, most patients with TNBC will experience early relapse. However, 1 in 5 patients with TNBC will demonstrate complete pathological response (pCR) to chemotherapy and will remain disease free for many years. GATA-3 is a protein normally expressed in breast tissue in response to estrogen stimulation although it is still present in a subset of TNBC, possibly under the influence of the androgen receptor (AR). Prior studies have suggested that GATA-3 expression may be a negative predictor for pCR in breast cancer although it is unclear if these results apply to TNBC. In this study, we examine for the first time the relationship of the expression of GATA in both AR positive and negative TNBC.
Design: All patients diagnosed at The Ottawa Hospital between January 2005 and March 2014 with TNBC who underwent surgery for locally advanced breast cancer after neoadjuvant chemotherapy were identified. Immunohistochemical analysis was performed for GATA-3 and AR. Both were scored using a composite of staining intensity and percentage cells stained. The primary outcome was pCR, defined as the absence of invasive disease in both breast and lymph nodes. Clinicopathological parameters and the expression of GATA-3/AR were compared in patients with and without pCR.
Results: Twenty-four patients were included in this study. The median age at diagnosis was 48 years (range, 30–70 years) and the median time of follow-up was 17.5 months (range, 1–88 months). Prior to chemotherapy the median tumour size was 54 mm (range, 8–130 mm); 18 patients had lymph node positive disease. Seven patients achieved pCR. There was no difference in the pre-chemotherapy tumour size (44 ± 28 mm vs. 54 ± 30 mm; p=0.764) or lymph node status (86% vs. 71%; p=0.629) between patients with and without pCR. GATA-3 expression was present in 20 cases (83%) while AR was present in 6 cases (25%). No AR expression was seen in 15 cases (63%) with GATA-3 positivity. There was no difference in either GATA-3 (4.3 ± 2.7 vs. 3.6 ± 2.5; p=0.549) or AR (1.4 ± 2.5 vs. 1.1 ± 2.4; p=0.778) expression between patients with and without pCR.
Conclusions: GATA-3 expression is frequent in TNBC even in the absence of AR expression. In contrast to previous studies expression of GATA-3 or AR is not associated with pCR after neoadjuvant chemotherapy in TNBC.

283 High-Grade Neuroendocrine Transformation of Post-Treatment Conventional Breast Carcinoma

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Background: Primary mammary high grade neuroendocrine carcinoma (HGNEC) is a rare entity and typically refers to *de novo* HGNEC of the breast. We recently identified cases of HGNEC arising at metastatic sites in patients with previously treated conventional breast carcinoma, analogous to high grade neuroendocrine transformation of post-treatment prostatic and lung adenocarcinoma.
Design: Four cases of HGNEC arising at metastatic sites in female patients with a history (hx) of conventional breast carcinoma (CBC) were identified during routine pathology practice at our institution. A diagnosis of HGNEC was only rendered if established morphologic and immunophenotypic criteria were met. Mammary origin was determined using clinical, radiologic, and pathologic correlation.
Results: All patients were female, aged 30-65 at the time of HGNEC diagnosis. Three were never smokers and 1 had a 26 pack-year smoking hx. All patients had a hx of prior CBC (ductal, NOS, grade 3 in all cases) and received chemotherapy and hormone therapy in the interval (mean 2.5 years; range 1-12) prior to developing metastatic HGNEC. Metastatic sites included the lungs (2 cases), mediastinum (1 case), and retroperitoneum (1 case). HGNEC showed small cell morphology in 2 cases and large cell in the others. By IHC, HGNEC showed the following results: All were positive for at least one neuroendocrine marker (synaptophysin, chromogranin, CD56); 2 were strongly and diffusely positive for ER; 2 maintained their original ER/PR/HER2 IHC profile following transformation; 2/2 cases were GATA3+; 1/1 was BRST2+; 1/2 was mammaglobin+; 1/2 was TTF-1+.
Conclusions: Whereas high grade neuroendocrine transformation following treatment is well-documented for both prostatic and lung adenocarcinoma, to the best of our knowledge, this is the first series describing the phenomenon in breast carcinoma. The propensity of these tumors to metastasize to thoracic sites has likely obscured their mammary origin in the past. Further studies are in progress to delineate the molecular profile of mammary HGNEC arising in the post-treatment setting and its relationship to conventional breast carcinoma.

284 Sequencing Analysis of Triple Negative Breast Carcinoma (TNBC) With a Large Central Acellular Zone (LCAZ)

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Background: Triple negative breast cancer (TNBC) with a large central acellular zone (LCAZ) is a previously described, rare, aggressive form of TNBC. The landscape of somatic genetic alterations of LCAZ is largely unknown, and it is unclear whether it differs from that of TNBC without LCAZ morphology.
Design: We searched our database for TNBCs treated at our center between 2002-2013 for which either frozen or FFPE matched tumor and normal tissue was available. Cases were centrally reviewed by 3 pathologists and cases consistent with a diagnosis of LCAZ were identified. Criteria for LCAZ included a centrally located hyalinized acellular zone representing ³30% of tumor area and not associated with coagulative necrosis, squamous debris, or matrix production. DNA was extracted from paired tumor (>75% tumor content) and normal samples and subjected to high depth massively parallel sequencing with the IMPACT platform, targeting all exons of 280 cancer related genes. Somatic single nucleotide variants were detected by MuTect and insertions/deletions by Somatic Indel Detector. Missense mutations flagged by any 2 of CHASM, FATHMM, Mutation Assessor, or Mutation Taster/Polyphen-2 were considered potentially pathogenic.
Results: A total of652 cases of TNBCs were reviewed. Among these, 18 cases meeting criteria for LCAZ with available matched tumor and normal tissue were identified. All patients were female, aged 28-78 years (median 57.5). Two patients (11%) had *BRCA1* germline mutations. Median tumor size was 2.1 cm (0.7-3.7). Five patients (28%) had lymph node metastases at presentation. The median follow up interval was 41 months (10-150). Five patients (28%) developed distant metastases (lung, brain, and bone) and 4 patients (22%) died of disease within 5 years of diagnosis. Sequencing analysis was completed for 6 cases with frozen tissue available. The average number of somatic mutations per case was 2.5 (1-4). *TP53* was the most frequently mutated gene in LCAZ, present in 4 of 6 (67%) cases. Somatic genetic alterations targeting genes pertaining to the PI3K pathway were found in all LCAZ analyzed (mutations: *PIK3CA, PIK3CB, PIK3R1, PTEN;* gains: *PIK3C2G, PIK3CB)*. Analyses of the remaining 12 LCAZ cases are ongoing.
Conclusions: Tumors with LCAZ morphology are characterized by recurrent *TP53* mutations and somatic genetic alterations affecting the PI3K pathway. The latter has

potential therapeutic implications and suggests a possible connection to metaplastic carcinomas, which, among the TNBC subtypes, have also been shown to be enriched for PI3K pathway alterations.

285 Comparison of PHH3, Ki-67 and H&E Mitotic Count in Invasive Breast Carcinoma

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Background: Modified Bloom and Richardson (mBR) grading is essential in the histologic evaluation of invasive breast carcinoma. Mitotic count on H&E-stained slides, a component of the mBR, is especially significant as it correlates strongly with progression-free survival. However, mitotic count is the most common reason for discordance in mBR scoring between pathologists. The Ki-67 proliferation index is a routine immunohistochemical (IHC) biomarker used in the evaluation of breast carcinoma, but is not specific for mitotic activity. Recently, the IHC marker antiphosphohistone-H3 (PHH3) has been proposed as potential surrogate marker for mitotic count. This study examines the differences between H&E mitotic count, PHH3 mitotic count, and Ki-67 index.

Design: Retrospective consecutive invasive breast carcinoma cases from April 2013-August 2014 were examined. PHH3 mitotic counts were assigned a mitotic grade using mBR criteria. To compare the Ki-67 index with H&E and PHH3 mitotic scores, the Ki-67 index was categorized into a three-grade system: < 10% (low), ≥ 10-<20% (intermediate), and ≥ 20% (high).

Results: A total of 451 cases were evaluated. PHH3 IHC mitotic count versus H&E mitotic count changed mBR scores in 24% of cases, upgrading in 22% and downgrading in 1%.

mBR changes/ total cases	Upgrade total	Upgrade G1-G2	Upgrade G2-G3	Upgrade G1-G3	Down-grade Total	Down-grade G2-G1	Down-grade G3-G2
107/451	101	53	41	7	6	2	4
24%	22%	12%	9%	2%	1%		

G1: grade 1, G2: grade 2, and G3: grade 3

A total of 431 cases had both Ki-67 and PHH3 IHC stains available for comparison. Both H&E mitotic grade and PHH3 mitotic grade correlated with Ki-67 in 51% of cases. Ki-67 correlated with PHH3 mitotic grade alone in 19% of cases and correlated with H&E mitotic grade alone in 8% of cases.

Conclusions: PHH3 stain facilitates the identification of mitotic figures and can be used as potential biomarker to enhance mBR assessment in the evaluation of invasive breast carcinoma. In our study, PHH3 was superior than H&E mitotic count and by changing mBR score by 24% and had a stronger correlation with the Ki-67 index.

286 Upgrade Rates on Surgical Excision of Atypical Glandular Breast Lesions Seen in Core Needle Biopsy

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Background: The clinical management of flat epithelial atypia (FEA) diagnosed by core needle biopsy (CNB) remains controversial. Upgrades to ductal carcinoma in situ (DCIS) and/or invasive carcinoma ranges from 0-23% on excisional biopsy in recent literature. The aim of this study is to assess surgical excision upgrade rate in FEA alone and other associated atypical lesions.

Design: PowerPath database was searched from the period of 1/1/2003 to 6/30/2014 with all breast CNB diagnosis of FEA alone, FEA with lobular neoplasia (LN), FEA with atypical ductal hyperplasia (ADH), ADH alone, and ADH bordering on DCIS. Excision pathology results were recorded.

Results: A total of 5,749 CNB were done; 2,589 by stereotactic, 2,912 by US and 248 by MRI guided respectively. A total number of FEA, FEA with LN, ADH with FEA, ADH and ADH bordering on DCIS were 97 (1.7%), 9 (0.02%), 99 (1.7%), 289 (4 %) and 18 (0.3%) respectively. Upon excision, upgrades were seen in 10% for FEA, 22% for FEA with LN, 15% for FEA with ADH, 24% for ADH alone and 61% for ADH bordering on low grade DCIS. Atypia on excision consists of ADH, FEA and LN (lobular carcinoma in situ and/or atypical lobular hyperplasia).

CNB	% case	Exc rate	Upgrade	Benign	Atypia	No F/U
FEA	1.7%	79%	10%	20%	47%	21%
FEA + LN	0.02%	78%	22%	11%	55%	22%
FEA +ADH	1.7%	77%	15%	8%	54%	23%
ADH	4.0%	80%	24%	22%	34%	20%
ADH bordering DCIS	0.3%	100%	61%	5%	33%	0

Conclusions: If CNB showed FEA alone, then the chances of malignancy was 10% on the final excision, and therefore, excision is recommended. Similarly, FEA admixed with LN or ADH on CNB should be excised. ADH alone and ADH bordering on DCIS require excision as previously recommended in literature.

287 Utility of Ki67 Expression in Subtyping Phyllodes Tumors of the Breast on Core Needle Biopsy and Excision Specimens

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Background: Phyllodes tumors (PT) are rare breast neoplasms; distinction among benign, borderline and malignant subtypes has therapeutic and prognostic implications. The association between Ki67 expression and PT subtypes has not been well studied. Thus our aim was to examine Ki67 expression in the preoperative core needle biopsy (CNB) and excision for correlation with final diagnosis and as a predictive marker of malignancy.

Design: We studied all patients diagnosed with a PT who had a preoperative CNB between 6/2000-3/2012. Ki67 quantification was performed via manual counting of stromal cells in both CNB and excision specimens. Final classification as benign, borderline, or malignant was based on WHO criteria as assessed in the excision specimens and correlated with Ki67 values. Receiver operator characteristic analysis was used to determine the best cut off point for distinguishing among categories.

Results: 30 patients had a benign, 11 a borderline and 6 a malignant PT. Ki67 expression was higher among the borderline/malignant vs. benign PT (median 15% vs 3.5%, p=0.0001 for CNBs; 20% vs 5%, p=0.0002, for excisions). For CNB, 7 (23%) of 30 benign PT had ki67 >=10%, while 3 (17.6%) of 17 borderline/ malignant had Ki67 <10%. For excision, 9 (30%) of 30 benign PT had ki67 >=10%, while 2 (11.7%) of 17 borderline/ malignant had Ki67 <10%. Area under the curve was 0.86 (95% CI 0.75 to 0.96). For CNB, a Ki-67 cut-off of >= 10% for predicting borderline/malignant PT resulted in a sensitivity of 81.3% and specificity of 75%, while for excision, this cutoff corresponded to a sensitivity of 88.2% and specificity of 67.9%. The correlation between CNB and excision Ki67 was 0.66.

Conclusions: We found significantly greater Ki67 expression among borderline/ malignant than benign PTs, in both CNB and excision specimens. A cut off point of 10% provides a good sensitivity and specificity in distinguishing between the two diagnostic categories. Ki67 could be used both preoperatively and after resection as a further discriminator in categorizing phyllodes tumors.

288 Integration of EGFR and CK5/6 Immunoexpression in Predicting Survival of Triple-Negative Breast Cancer

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Background: TNBCs, defined as those lacking ER, PR, and HER2 expression, have been shown to be molecularly heterogeneous by gene expression analyses with a majority expressing basal markers. These basal-like breast cancers (BLBC) are associated with shorter survival and poor prognosis. Immunoexpression of CK5/6 and EGFR has been used to clinically identify BLBC, however the heterogeneity in staining and the absence of defined cutoffs limit the use of such IHC-based surrogates. This study identifies the prognostic value of EGFR and CK5/6 for systematic survival prediction, and develops appropriate cutoff values for predicting survival in TNBC patients.

Design: 200 consecutive primary resections of TNBCs between 2008 and 2012 with clinical follow up were retrieved. IHC staining for EGFR, CK5/6, Ki-67 and p53 was evaluated on whole sections. All patients had lumpectomy or radical mastectomy as primary treatment. Tumor size, grade, lymph node status and expression of EGFR, CK5/6, Ki67 and p53 were correlated with disease-free survival (DFS) using a univariate Cox analysis, Kaplan-Meier (KM) curves and log-rank tests. The systematic prognostic value integrated with EGFR and CK5/6 was evaluated by a multivariate Cox model.

Results: The prognostic value of EGFR and CK5/6 at different cutoff percentiles was assessed using log-rank tests and KM curves. A 15% cutoff point was selected for EGFR (p=0.003), and 50% for CK5/6 (p=0.016). Results of univariate Cox analysis indicated that DFS was significantly associated pathological T stage (p=0.002), N stage (p=0.001), EGFR (p=0.001), and CK5/6 (p=0.018). A multivariate cox model was built for predicting DFS with five variables: EGFR (p=0.004), T stage (p=0.0003), N stage (p=0.003), CK5/6 (p=0.007), and Ki-67 (p=0.032). Based on the derived model, patients were categorized into low and high-risk groups separated by the 50th centiles of prognostic indexes. The 50-month DFS rates in low-risk and high-risk groups were 94.4% and 56.8%, respectively (p<<0.001).

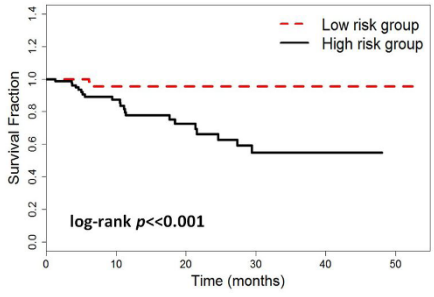

Figure 1. Survival fractions predicted by multivariate cox model for low risk and high risk patient groups.

Conclusions: This study shows that integrated prognostic analysis of basal-like biomarkers would improve the accuracy of predicting survival outcome, and further help identify patients at higher risk who may benefit from targeted treatments.

289 Frequency of cMet Aberration By FISH and Immunohistochemistry Studies in Triple Negative Breast Cancers

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Background: c-MET (mesenchymal-epithelial transition factor gene) is a proto-oncogene on chromosome 7q31 activating several downstream signaling pathways. High c-MET expression has been shown to be associated with poor prognosis in hormonal receptor positive and Her2 positive breast cancers. However, its incidence in triple negative breast cancers has not been explicitly explored.

Design: Archival paraffin-embedded tissue blocks were selected from one hundred and six female patients with diagnosis of triple negative invasive breast carcinoma that had surgeries at the University of Texas-MD Anderson Cancer Center. Medium follow up is 69.4 months (range 9-317 months). Expression of c-MET was assessed by IHC using rabbit monoclonal anti-total c-Met antibody (SP44 from Ventana). Staining intensity was scored on a scale of 0, 1+, 2+ and 3+. Samples that scored ≥ 2+ in ≥ 50% of tumor cells were considered to be positive for c-MET overexpression. FISH analysis was performed using MET (7q31) specific FISH probe (Kreatech). The chromosome 7 satellite enumeration (SE) probe is used for control. Sixty tumor cells were counted for each sample. c-MET gene copy numbers ≥ 4 per cell or c-MET/CEP7 ratio ³2 is used as cut-off for c-MET amplification.

Results: c-MET was overexpressed in 13 out of 103 cases by IHC. c-MET was amplified in 2 (c-MET/CEP7 ratio ³2) and 3 (≥ 4 c-MET copies per tumor cell) out of 99 cases by FISH. Only one case met both criteria for c-MET amplification by copy number and c-MET/CEP7 ratio. Not a single case was identified to be over-expressed by IHC and amplified by FISH at the same time.

At the time of the analysis, 23 women (21.7%) had died, and 20 (18.9%) had experienced a recurrence. However, neither of c-MET overexpression or amplification is statistically significant for the relapse free survival (RFS) or overall survival (OS), after being adjusted to age, tumor size, nodal status, and adjuvant chemotherapy drugs through multivariable Cox proportional hazard analysis. In addition, the status of c-MET does not correlate with IHC status of ER, PR and Her2 status of the tumors.

Conclusions: In this study, the frequency of c-MET dysregulation, including overexpression and amplification was assessed in a small cohort of triple negative breast cancers and the correlation of survival data was also explored. The insignificance of either c-MET overexpression by IHC and amplification by FISH correlating to the survival data might be due to the low incidence of c-MET aberration in this specific subtype of breast cancers.

290 The Spectrums of Cannabinoid Receptor Type 2 (CB2) Expression in Human Breast: A Pilot Morphological-Immunohistochemical Study

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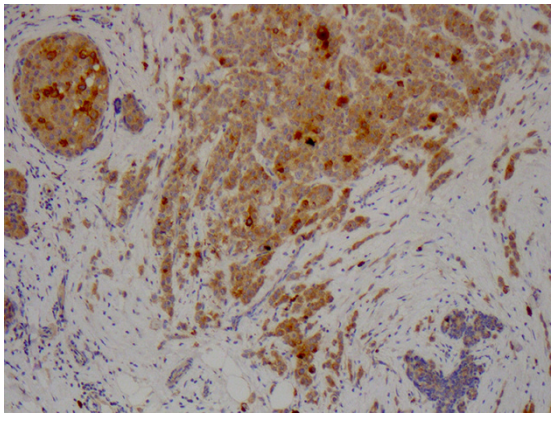
Background: Cannabinoids receptors (CB) are a class of G protein coupled receptors mainly expressed in central nerve system (CB_1) and in immune system (CB_2). Recently, CB modulation has been found to suppress tumor growth and metastasis through direct and indirect pathways. In particularly, CB_2, the non-psychotropic type receptor, has been shown to be altered in breast cancer by both *in vitro* and *in vivo* studies. However, the spectrum of CB_2 expression in human breast cancer has not been studied on histologic material.

Design: Series of 36 cases of various breast benign and malignant lesions (including 5 fibrocystic changes, 5 invasive and in-situ lobular carcinomas; 26 invasive and in-situ ductal carcinoma) were subjected to the study. Immunohistochemical staining was performed on paraffin sections with Leica Bond-III instrument with rabbit polyclonal anti-CB_2 (1:50, Thermo Scientific). CB_2 immunoreactivity was evaluated semi-quantitatively as: negative; equivocal background expression (1+); granular cytoplasmic expression in 1-5% cells (2+); in 5-20% cells (3+); strong granular and diffuse cytoplasmic stain in >20% cells (4+).

Results: Benign human breast tissue and lesions show either negative or revealing background staining. Invasive ductal carcinoma exhibits marked CB_2 up-regulation (30.8% with 4+, 19.2 % with 3+ and 15.4 % with 2+). The CB_2 expression level seems to be associated with the tumor grade (4+ in 6/7 high grade tumors and 1+ in 2/3 low grade tumors). Similarly, intraductal carcinoma shows increased CB_2 expression. CB_2 expression remains low in invasive or in-situ lobular carcinoma (all cases with 1+).



Conclusions: Our study indicates that CB_2 expression is frequently up-regulated in human breast ductal carcinoma. The paradoxical up-regulation of CB_2 was previously reported in prostate cancer and is poorly understood. The observation suggests that CB_2 play a role in ductal carcinoma progression and be potentially used as therapeutic target for breast cancer. Further investigation of role of CB_2 in breast cancer is warranted.

291 The Impact of 2013 Updated ASCO/CAP HER2 Guideline on the Diagnosis and Management of HER2 Positive Breast Cancer: A Single Center Retrospective Study

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Background: In 2013, ASCO/CAP updated its 2007 HER2 testing guidelines for breast cancer. The purpose of this study was to determine the impact of revised guidelines in clinical practice.

Design: We retrospectively analyzed prevalence of HER2 alterations using both 2007 and 2013 guidelines in a cohort of 1739 breast cancer cases who received reflex FISH assay at our institution between 2007 and 2013 (cases with IHC score 1+, 2+, some 0 with discordant histological features and some 3+ with heterogeneous staining pattern).

Results: Using 2007 guidelines, 186 (10.7%) cases were classified as HER2 positive whereas using 2013 guidelines 255 (14.7%) cases were classified as HER2 positive ($p<0.01$). A total of 69 cases, equivocal by 2007 guidelines (4% of all cases and 10% of all equivocal cases) were converted to HER2 positive by 2013 guidelines. Sixty-three of these 69 cases changed from HER2 equivocal category to HER2 positive due to change in FISH amplification ratio cut-off from 2.2 to 2.0. Remaining 6 cases had FISH ratio <2.0 but IHC score 3+ in 10-30% tumor cells. None of the HER2 negative cases changed to HER2 positive category by new guidelines. Six out of 66 cases (9.1%) in the group with HER2 FISH ratio 2.0~2.2 exhibit 3+ IHC staining, compared to only 1 case (1.2%) in the group with HER2 ratio between 1.8 to 2.0 ($p<0.01$). This supports stratification of the former group within HER2 positive category in 2013 guidelines. The clinical management information was available for 43 of these 69 cases converted to HER2 positive according to 2013 guidelines and only 8 of them received Herceptin treatment. FISH test and IHC test were discordant in 55 cases (3.2%) when using 2013 guidelines, including 33 FISH positive (ratio³2.0) but IHC negative (0+ or 1+ in <10% of tumor cells), and 22 IHC positive (3+ in ³10% of tumor cells) but FISH negative (ratio <2.0) cases.

Conclusions: Implementation of 2013 guidelines significantly increases the prevalence of HER2 positive cases by 4% ($p<0.01$). Approximately 10% (69/581) of HER2 equivocal cases are converted to HER2 positive by 2013 guidelines. Failure to test 1+ IHC score cases with reflex FISH assay may miss some potentially treatable patients.

292 Metastastic Neoplasm To the Breast: Study of 210 Cases

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Background: Metastasis to the breast from other solid organs is a rare and unexpected event, and therefore can create diagnostic challenges. Many cases are misdiagnosed as primary breast carcinomas, resulting in inappropriate patient management. The purpose of the current study is to evaluate the clinical and pathologic features of metastatic neoplasms to breast, in order to identify parameters that might help to differentiate metastasis from primary mammary tumors.

Design: Metastatic tumors except hematopoietic malignancies to the breast were searched from the computerized Surgical Pathology files at our tertiary care hospital. A total of 210 cases were identified during the period from January, 2005 to May, 2014. Clinicopathologic features of those cases were retrospectively reviewed, including: Primary tumor type/site; duration between diagnosis of the patient's primary tumor and diagnosis of the breast metastasis; method of detection; unilateral, bilateral, or multifocal location of the breast metastasis; and original diagnosis of the breast specimen.

Results: Median patient age was 56 yr at the time of breast metastasis (range 13-85 yr), with a marked female predominance (92%). Primary tumors included melanoma (88, 42%), serous carcinoma (34, 16%, predominantly ovarian), neuroendocrine (28, 13%, predominantly from lung and gastrointestinal tract), sarcoma (21, 10%, predominantly leiomyosarcoma), and adenocarcinoma from various organs (21, 10%), among others. Most metastases were unilateral (199, 95%) and unifocal (183, 87%), and were detected by radiologic rather than physical examination (45% vs. 23%, respectively). Concurrent ipsilateral axillary metastasis occurred in 49 (23%) patients. Mean duration between primary diagnosis and breast metastasis was 2.2 yr (range 0-37 yr). In 34 (16%) cases, breast metastasis was the first clinical presentation of disease, and 13 (38%) of these case were initially misdiagnosed as breast primary. In contrast, with known history of other primary tumors, only 3 (2%) cases were misdiagnosed ($p<0.0001$).

Conclusions: Metastatic tumors share many features with breast primary carcinomas, including similar age, female predominance, unifocality, with similar rates of ipsilateral axillary nodal metastasis. However, cases of well circumscribed tumor, lack of in situ component, and ER/PR negativity on routine staining should raise the consideration of metastatic disease. Most importantly, careful review of the patient's clinical history can often lead to a correct diagnosis.